INSTITUTE OF MOLECULAR BIOLOGY AND GENETICS OF
NATIONAL ACADEMY OF SCIENCES OF UKRAINE

Materials of the 4th Conference of Young Scientists, dedicated to Illya Mechnikov 165th Anniversary (18 19 May 2010)

Collection of Theses

Institute of Molecular Biology and Genetics NAS of Ukraine, 2010

CHRONICLE AND INFORMATION

Zeolite nanoparticles for improvement of analytical characteristics of conductometric enzyme biosensor for urea determination

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Porous and composite materials have recently received much attention due to their application as components of bioselective elements of electrochemical biosensors. The aim of this work was to research influence of NH_4^+-sensitive zeolite on analytical characteristics of the conductometric enzyme biosensor for urea determination. Natural zeolite clinoptilolite used in this study is known to display perceptible preference for ammonium ions (over other small cations) and this unique property was exploited for selective ammonium removal from aqueous effluents. The powdered clinoptilolite was obtained from Societe Mediterraneenne des Zeolithes (France). Average zeolite particle size was 400 nm (90 % between 200 nm and 1.0 m). The sample was microporous with a specific surface area of about 100 m^2g^{-1}. The chemical composition of the unit cell formula was $(Na_{0.10}K_{0.57})$ $(Ca_{0.47}Mg_{0.15})$ $(Al_{1.97}Fe_{0.12})$ $(Si_{9.96}Ti_{0.02})O_{24}\cdot 7H_2O$. An increase in the biosensor sensitivity to ammonium ions could be assigned to good adhesion between the polymer and zeolite particles together with an efficient combination of a zeolite/urease membrane with a thin-film planar electrode. Urease based sensors that differ in the composition and arrangement of bioselective membranes and conditions of their immobilization were studied. Immobilization of zeolite-modified membranes on the surface of gold interdigitated electrodes was carried out by cross-linking with glutaraldehyde. The dynamic and linear ranges and operational stability of the developed biosensors were compared. In addition, the dependency of responses of urease biosensors on pH values and buffer capacity of the working solution was obtained. The results showed that zeolite nanoparticles exhibited a capacity for expansion of the dynamic and linear ranges of all types of bioselective membranes for urea determination. The study showed a possibility to modulate both linear and dynamic range of the biosensor based on the urease/BSA membrane, using different variants of incorporation of ammonium-sensitive zeolite particles into the bioselective membrane.

Computational model of full length cytochrome P450 2E1 bound to the phosphatidylcholine membrane

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Human cytochrome P450 2E1 (CYP2E1) belongs to the membrane-associated hemeproteins – a cytochrome P450-monooxigenase superfamily which performs oxidation of endogenous and exogenous compounds in cells. CYP2E1 molecule has a pyramidal shape; its base is associated with endoplasmic reticulum membrane through hydrophobic N-terminus. This complicates experimental investigation of structural and functional characteristics of the native enzyme, therefore, CYP2E1 spatial structure is studied mainly by computer methods. Recent researches in genetic engineering have allowed getting and purifying soluble forms of cytochrome P450 isoforms, which natively are hydrophobic, thus making their NMR study possible. Thereby, 3d-structures of CYP2E1, obtained both experimentally and with homology model, missed N-terminal hydrophobic anchor, which may affect the enzyme spatial geometry. The purpose of our work was to create native-like 3d-structure of full length CYP2E1of bound to membrane using computational methods. First, we created a computer model of an -helix hydrophobic anchor (30 aa) using ArgusLab program. This -helix model and the computational model of CYP2E1 spatial structure previously optimized by us were used as the templates at I-TASSER web server (http://zhanglab.ccmb.med.umich.edu) to build a full-size model of human cytochrome P450 2E1. Using the program VMD we created the bilayer phosphatidylcholine – POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine) membrane. The enzyme molecule was oriented against the membrane so that the hydrophobic anchor dived in it. To optimize the resulting membrane-protein complex, a short (500 ps) molecular dynamics simulation was made in water environment using NAMD program. Thus, we received the computer model of the spatial structure of human cytochrome P450 2E1 associated with the membrane. This model allows us to predict and evaluate more correctly the enzyme – ligands interactions.

Molecular basis of transition mutations induced by 2-aminopurine: quantum-chemical study

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Application of simple physico-chemical principles for clarification of spontaneous and induced mutagenesis is one of the important tendencies in modern biophysical investigations. **Aim**. Elementary molecular mechanisms of mutagenic action of 2-aminopurine (2amPur), considered to be one of the stumbling stones of this problem, still remains obscure, therefore the aim of our work was to fill this gap. **Methods**. Quantum-chemical modeling on the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory. **Results**. On the basis of quantum-chemical models of the spontaneous transitions of insertion and replication, proposed by us earlier [1, 2], we have shown for the first time: inadequacy of a hypothetical ionization mechanism of the 2amPur mutagenic action; much higher frequency of the replication mispairs induced by 2amPur than the inclusion mispairs in the 2amPur Cyt > 2amPur Cyt* pathway (*marks a mutagenic tautomeric form); the replication errors induced by 2amPur are realized in the pathway 2amPur Thy > 2amPur Thy*: at that 2amPur Thy* population exceeds the 2amPur* Thy population under normal conditions by 9.38 10^5 times. **Conlusions**. The results obtained allow us to explain well known experimental data.

1. Brovarets O. O., Hovorun D. M. Physico-chemical mechanism of the wobble DNA base pairs Gua·Thy and Ade·Cyt transition into the mismatched base pairs Gua*·Thy and Ade·Cyt* formed by the mutagenic tautomers // Ukrainica Bioorganica Acta.–2009.–**8**, N 2.–P. 12–18.

2. Brovarets O. O., Hovorun D. M. The novel physico-chemical mechanism of the Watson-Crick base pair Ade·Thy transformation to the mispairs involving rare tautomers Ade*·Thy and Ade·Thy* // Ukrainica Bioorganica Acta.– 2010.–**8**, N 1.–P. 3–9.

Transduction of murine fibroblast cell culture by recombinant baculoviruses containing different regulatory elements

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At present a possibility to use both mesenchymal stem cells and mature fibroblasts as cell vectors is actively studied for the purposes of anticancer therapy. As for human fibroblasts, their immunoregulatory properties are known to resemble those of mesenchymal stem cells and, therefore, they could be used for the development of cell therapy. We suggested that in model experiments fetal murine fibroblasts could be used as an advantageous source for the cell vectors generation instead of sophisticated mesenchymal stem cells. As an expression vector we used a well-studied baculovirus vector system providing an effective delivery of genes into mammalian cells while being inert for the cell because of the lack of self-replication in these cells and the ability to destruct cell. It is known that the transduction efficacy depends on many factors including the activity of the promoter used. **Aim**. We aimed at a study of primary fetal murine fibroblasts transduction efficacy using two recombinant baculovirus constructions in which a reporter gene *eGfp* was regulated by two different promoters: CMV (Ac-CMV-GFP virus) and that of a chicken -actin (Ac-CAG-GFP virus). As a control we studied efficiency of transduction by these recombinant viruses of HEK293 and HeLa cells. **Methods**. Recombinant baculoviruses were constructed on the basis of AMNPV virus using the Bac-to-Bac (Invitrogen) system. Transduction efficiency and average fluorescence intensity were measured using flow cytometer Coulter Epics XL. The primary culture of fetal murine fibroblasts was obtained from soft tissues of 14-day embryos using tissues enzymatic (C57Fb) desegregation. **Results**. For both constructions a high efficacy of transduction was observed that correlated with the virus dose used. For Ac-CAG-GFP virus at the doses of 20, 200 and 500 moi it was 33.7 ± 1.2 %, 69.6 ± 3.8 %, 75.8 ± 2.7 %, respectively; for Ac-CMV-GFP virus and at the same doses it was 13.3 ± 2.9 %, 59.1 ± 13.1 %, 85.2 ± 8.8 %, respectively. Interestingly, at the lowest doses (20 moi) the constructions differed more obviously, the Ac-CAG-GFP being more active, while at the high doses this difference was not practically observed. Study on transduced cells fluorescence duration showed that the number of fluorescent cells at any dose is gradually decreased in cases of both constructions. However, in case of Ac-CAG-GFP there were more intensely fluorescent cells and some cells retained fluorescence for 20 days while only 13 days for the Ac-CMC-GFP construction. **Conclusions**. Fetal murine fibroblasts obtained were able to be actively transduced using baculoviral vectors. The recombinant construction Ac-CAG-GFP provided more pronounced and prolonged expression of the reporter gene used in the experiments. The dose of construction used to get the optimal results of transduction efficacy and inert for cell viability *in vitro* was 200 moi.

Distinct structural features of mRNAs coding A1 and A2 isoforms of translation elongation factor EF1

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Aim. The main function of eEF1A is the delivery of aminoacyl-tRNA to the A site of the ribosome during the elongation step of protein biosynthesis. It is known that appearance of eEF1A2 in non-specific tissue is related to malignant transformation of the cell. eEF1A1 is proapoptotic while eEF1A2 possesses antiapoptotic, pro-oncogenic properties. Despite a high similarity in their coding regions, the mRNAs for two eEF1A isoforms are appreciably different in their 5' and 3' untranslated regions (UTR). This fact suggests a possibility of differential post-transcriptional regulation of the isoforms. The work is aimed to disclose and compare the *cis*-regulatory elements in the A1 and A2 mRNA UTR's as well as to determine the features of their functioning *in vivo*. **Methods**. The programs miRANDA, PicTar, TargetScan 5.0, microT 3.0, PITA were utilized to detect specific motifs in UTR's. RNAfold program was used for the RNA secondary structure prediction. For detection of the conserved RNA secondary structures, we used RNalifold. PCR and RT-PCR were used to obtain 3'UTRs of eEF1A2 and eEF1A1 in preparative amounts for further cloning into reporter plasmids. **Results**. *In silico* analysis showed that 5'UTR of eEF1A2 lacks terminal *oligopyrimidine* tract which is well-known attribute of eEF1A1 mRNA. This fact indicates that the translation of eEF1A2 is not regulated by the mTOR signal cascade. Additionally, 5'UTR of eEF1A2 is more structured than that of eEF1A1. One may suggest the initiation process could be slower for eEF1A2 than for eEF1A1 mRNAs.The conserved site for binding SAM68 protein was detected in 3'UTR of eEF1A2. The site is located in the unstructured region that is in agreement with literature data. Moreover, 3'UTRs of eEF1A1 and eEF1A2 are found to harbor multiple binding sites for different microRNAs. To validate experimentally the predicted microRNAs binding sites, 3'UTRs of eEF1A1 and eEF1A2 mRNAs were cloned into *pGL4.13* reporter vector for subsequent investigation in dual-luciferase reporter system. **Conclusions**. The obtained *in silico* results show that eEF1A1 and eEF1A2 mRNAs might be differently regulated at the posttranscriptional level suggesting they could be involved in different cellular processes. The next step is to validate the data obtained experimentally.

Structure basis of amino acid specificity maintenance by *Enterococcus faecalis* prolyl-tRNA synthetase

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Aim. Some of aminoacyl-tRNA synthetases (ARSases) possess special function for correction of mistakes occurring during amino acid recognition, named «editing». Pre-transfer editing corresponds to the hydrolysis of mis synthesized aminoacyl adenylates, and post-transfer editing – to the hydrolysis of mis synthesized aminoacyl-tRNAs. Editing mechanisms of the second structural class ARSases are less investigated. Especially it concerns prolyl-tRNA synthetase (ProRS). ProRS is able to carry out pre-transfer, as well as post-transfer editing against alanine. Previously we confirmed the localization of post-transfer editing active center of prokaryote *E. faecalis* ProRS (ProRS*Ef*), and showed that pre-transfer editing occured in the aminoacylating active center. It was also shown pre-transfer editing acceleration in the presence of $tRNA^{Pro}$, and the key role of hydroxyl groups of 3'-terminal adenosine in this process. The aim of this work was to study the distribution of authority between these 2'- and 3'-hydroxyl groups, and to extend our knowledge about a role of editing domain amino acid residues in post-transfer editing. **Methods**. 2'-deoxy and 3'-deoxy $tRNA^{Pro}$ were obtained by enzymatic approach. Site-directed mutagenesis was carried out by QuickChange method (Stratagene). For editing activity rate estimation, the test based on ATP hydrolysis in the presence of uncognate amino acid, and the test based on alanyl-$tRNA^{AlaPro}$ deacylation, were used. **Results**. We have shown that tRNA-dependent editing against alanine completely depends on both 2'- and 3'-hydroxyl groups of 3'-terminal adenosine. Influence of I333A ProRS*Ef* mutation on post-transfer editing rate was revealed. The decrease of tRNA-dependent editing specificity due to I263A and I278A mutations was also shown. Rather strong tRNA-dependent editing against proline by H369A mutant form, shown earlier on *Escherichia coli* ProRS, was confirmed on analogous ProRS*Ef* mutant form H366A. **Conclusions**. Critical importance of both 3' and 2'-hydroxyl groups of tRNA 3'-terminal adenosine for editing activity has been shown. The data on ProRS*Ef* mutant form editing activity are consistent with the existing computer model of ProRS*Ef*–alanyl-$tRNA^{Pro}$ complex in editing conformation.

Institute of Molecular Biology and Genetics NAS of Ukraine, 2010

Identification of human protein kinase ASK1 inhibitors among 2-thioxo-thiazolidin-4-ones derivatives

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Protein kinase ASK1 plays a key role in cell differentiation, aging and apoptosis. The increased activity of the kinase is linked to several pathologies, including neurodegenerative, cardiovascular, liver diseases and cancer. The ASK1 inhibitors may be important compounds for development of clinical agents. None of the small molecule inhibitors of ASK1 have been reported in scientific literature so far. In our early experiments we had identified that derivatives of 2-thioxo-thiazolidin-4-one exhibited the inhibitory activity against ASK1. The most active compound inhibited ASK1 in a dose-dependent manner with IC_{50} 2 M. The main aims of our research are to identify small molecule inhibitors of ASK1 among derivatives of 2-thioxo-thiazolidin-4-one and to predict a binding mode for these ligands. In this study, for development of novel potent and selective protein kinase inhibitors we have performed screening program, using both *in silico* and conventional approaches. For receptor-ligand flexible docking the system based on DOCK 4.0 package was used. We carried out the virtual screening experiments using the database containing 8425 derivatives of 2-thioxo-thiazolidin-4-one. Twenty promising candidates were taken for the kinase assay analysis. Three compounds displayed activity in the *in vitro* ASK1 inhibition assay. The most active compound inhibited ASK1 in a dose-dependent manner with K_i 250 nM. The Lineweaver-Burk plots demonstrated competitive character of the inhibition. Selectivity of the compound was estimated in the reactions with ASK1, Aurora, ROCK, FGFR, c-Met, JNK3, CK2, Tie2 human kinases and the specific substrates for each individual enzyme. Our molecular docking investigations have clearly demonstrated that the compounds of this class display a very good steric and chemical complementarity with the ATP binding cavity. The peculiarity of binding mode of the most active compound in comparison with other nineteen tested compounds is its ability to bind simultaneously the part of the kinase domain, known as the hinge region, that links the distinct N- and C-terminal lobes, and the phosphate-binding region of the ATP-binding cleft. The core structure of this compound can be used for further optimization and discovering more potent and selective inhibitors of ASK1.

Search and design of novel protein kinase CK2 inhibitors

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A crucial role of protein kinases in cell signaling, gene expression, and metabolic regulation is highlighted by the fact that nowadays this family of enzymes is the second of most important drug targets. CK2, one of the first protein kinases ever discovered, is Ser/Thr protein kinase. This ubiquitous enzyme is among the most highly conserved molecules throughout evolution and is present in every cell, both in the cytosol and the nucleus, at a strictly regulated level depending on the cell type. CK2 is involved in many cellular processes such as cell cycle regulation, circadian rhythms, gene expression, cell growth and differentiation, embryogenesis, apoptosis, transcription and viral infection. Abnormal levels of the enzyme detected in several types of cancer, including prostate, mammary gland, lung, kidney, and head and neck, suggest the involvement of CK2 in tumorogenesis. CK2 has also a crucial role in the transduction of survival signals, protecting the cell against stress events. The observation of CK2 deregulations in various pathological processes suggests that CK2 inhibitors may have a therapeutic value, particularly as anticancer and antiviral drugs. Therefore, the aim of this work is search and design of novel CK2 inhibitors. Here we present the new CK2 inhibitors belonging to the class of 4-thio-thieno [2,3-d]pyrimidine acids derivatives. We have identified this class of protein kinase inhibitors by high-throughput molecular docking of a compound collection in the ATP-binding site of human CK2. 28 compounds were tested using the *in vitro* kinase assay (-^{32}P-ATP method). 20 of them showed the activity with IC_{50} less than 30 μM and 4 – less than 1 μM. The most active compound is 3-(5-p-Tolyl-thieno[2,3-d]pyrimidin-4-ylsulfanyl)-propionic acid (IC_{50} = 100 nM). We researched the binding mode of selected compounds and established the relationship between their chemical structure and biological activity. The most active compounds were also tested on a series of 7 purified protein kinases assayed in the presence of 10 μM inhibitor. The results of this research confirm the selectivity of 4-thio-thieno[2,3-d]pyrimidine derivatives towards CK2. Thus, the novel potent selective inhibitors of CK2 have been found. The results obtained can be used for further optimization of this class compounds.

Synthesis and optimization of 2-phenylisothiazolidin-3-one-1,1-dioxides as inhibitors of human protein kinase CK2

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Protein kinase CK2 (Casein Kinase 2) plays an important role in transduction and enhancement of various cell growth and metabolic signals. The observation of CK2 deregulation in various pathological processes suggests that CK2 inhibitors have a therapeutic value, particularly as antineoplastic and antiviral drugs. The aim of our work was a synthesis of new derivatives of 2-phenylisothiazolidin-3-one-1,1-dioxides and study of their inhibition activity toward kinase CK2. Potential inhibition activity of this compound was predicted by flexible docking (programm package DOCK 4.0). Synthesis of the predicted compound series was carried out using combinatorial synthesis techniques. Series of 19 compounds were tested *in vitro* for CK2 inhibitory activity. It was found that N-(3-acetylphenyl)-2-chloro-4-(4-methyl-1,1-dioxido-3-oxoisothiazolidin-2-yl)benzamide has IC_{50} value of 20 M. To perform further structure optimization other compounds of this class were synthesized and tested *in vitro*. Therefore, we suppose that the presented compounds is a promising class of novel CK2 inhibitors.

QSAR methodology for search of inhibitors of amyloid fibril formation

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Aggregation of proteins into insoluble amyloid fibrils plays a key role in development of a number of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. Thus, the obtaining of compounds capable to modulate protein fibrilization is of high biomedical importance. Aim of this work is to apply QSAR approach to the search of potential fibrilization inhibitors among representatives of flavones class. QSAR methodology as a section of ligand-based screening is widely used for searching ligands for biological targets in the absence of the information about a binding site structure, and also in case if to allocate a concrete, accurately defined site is not obviously possible. For search of potential inhibitors of amyloid fibrils formation the regression analysis with back propagation neural networks was used. The training set contained a series of known inhibitors belonging to the flavonoid class. The base which has been filtered according to Lipinsky rules contains 300 flavonoids. For the subsequent processing, 144 substances with the most drug-like abilities were chosen. Using back propagation neural network, a model with good regression characteristics has been received. According to this model, 10 hits have been chosen for *in vitro* tests. The recently developed cyanine-based fluorescent inhibitory assay demonstrated a noticeable effect on insuline fibrilization for 6 of 10 above mentioned compounds. To extend amount and diversity of active compounds, basing on the information obtained from our last results, we are planning to increase the amount of processed chemical classes.

Design of protein kinase CK2 inhibitors based on 3-substituted 2-quinolinon derivatives

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During the last decades a great interest of researchers has been attracted to the search of new protein kinase inhibitors as potential antiviral and anticarcinogenic drugs. Casein kinase 2 (CK2) has enhanced activity in a wide spectrum of tumours and is used by many viruses for phosphorylation of their inherent proteins and inhibition of cellular mechanisms of apoptosis, which allows considering CK2 as a target for creation of new drugs of directed action. As a result of computer design by the method of flexible doking (program package DOCK 4.0), 70 compounds most able to interact with the surface of the enzyme ATP-binding site were selected out of the virtual library of about 3000 3-substituted derivatives of quinoline-2. Among the compounds synthesized, a few inhibitors of CK2 with IC_{50} value of 16–50 M are found by the testing *in vitro* with -marked ATP. The predicted inhibiting activity was found in a series of 2-(7-metoxy-2-oxy-1,2-dihydroquinolin-3-il)-*N*-arylacetamides. The data obtained specify the direction of further chemical optimization of the inhibitor structure, which will be conducted in subsequent work.

Detection of DNA methylation of gene promoters in clear cell renal carcinomas

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Aim. Renal cell carcinoma (RCC) is the 10th of the most frequently detected types of cancer. The most common histological type of RCC is clear cell renal carcinomas (ccRCC) which are presented by 70 % of incidence. This type of renal cancer is characterized by strong resistance to chemical and radio therapies, but can be succesfully treated by surgical resection at initial stages. It was shown that change in DNA methylation is an early event in the process of cancer transformation for wide tissue range. Therefore, a study on epigenetic alteration in clear cell renal carcinomas at different stages is important for early detection of ccRCC at surgically curable stage. **Methods**. To perform massive search of genes with abnormal DNA methylation at chromosome 3 we have used a novel *NotI*-microarray technology. Further, verification of *NotI*-microarray results was done by MSP and bisulfite sequencing. **Results**. Checking by *NotI*-microarray has resulted in a set of genes with genetic or epigenetic changes. We have selected the genes which were changed in more than 30 % of samples. Among detected genes were *WNT7A* and *LRRC3B* genes. MSP analysis and bisulfite sequencing have shown hypermethylation of promoter regions of *WNT7A* and *LRRC3B* genes. **Conclusions**. We have obtained close correlation between the data obtained by conventional methods, MSP and bisulfite sequencing, and the results of *NotI*-microarray. In most cases the changes detected by *NotI*-microarray were presented by DNA hypermethylation.

Application of potentiometric urease biosensor for control of hemodialysis

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Urea is a main and final product of protein metabolism, its normal range in human serum is between 1.7 and 8.3 mM and can increase up to 100 mM under pathophysiological conditions. Urea is an important diagnostic substance in biological fluids. Its level often provides the information on the nutritional status of dialyzed patients. Moreover, urea is recognized as a marker of toxic compounds accumulation in patients with diminished renal functions. Chronic hemodialysis is a life-saving therapy for patients with strong renal disorders. The main goal of hemodialysis is removal of toxins from the patient. The monitoring of hemodialysis is the best way for biomedical evaluation of correctness and efficiency of this clinical treatment. Today colorimetric and spectrometric methods are most commonly used for determination of urea in biological samples. However, these methods are complex in application, have low selectivity, and require pretreatment of samples. **Aim**. The main goal of the work is development of the biosensor for detection of urea concentration in dialysate and blood serum. **Methods**. We propose for urea detection the potentiometric biosensor based on pH-sensitive field-effect transistors and immobilized urease developed in our laboratory. **Results**. The present research is focused on optimization of working characteristics of the developed biosensor for urea detection in dialysate and blood serum. The conditions of urease immobilization in bovine serum albumin in glutaraldehyde vapor were optimized. The detection range of the developed biosensor was determined to be 0.02–5 mM urea. The urea biosensor demonstrates good reproducibility and operational stability. Dependencies of the biosensor responses on ionic strength and buffer capacity of the analyzed sample were investigated. The calibration curve in dialysis liquid demonstrates the minimal detection limit for urea to be 0.02 mM. The biosensor was tested for urea determination in dialysate and blood serum. **Conclusions**. Highly sensitive and selective urea biosensor based on pH-sensitive field-effect transistors and immobilized urease is developed. Its application for urea detection in biological samples is demonstrated.

Search of the regulatory elements in 3'UTR of human Intersectin 1 mRNA

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Aim. Intersectin 1 is a multidomain adapter protein the gene of which encodes two isoforms – short (ITSN1-S) and long (ITSN1-L) and takes part in clathrin-mediated endocytosis, cell signaling and cytoskeleton reorganization. 3'Untranslated region (3'UTR) of intersectin mRNA, as well as 3'UTRs of mRNAs of many other genes, may contain target sites for different regulatory factors and thus could play an important role in post-transcriptional regulation of ITSN1 gene expression. The aim of our work was to identify and experimentally confirm potential target sites for different regulatory factors in 3'UTR region of ITSN1 mRNA. **Methods**. To generate deletion mutants of 3'UTR of ITSN1-S we have cloned different regions of ITSN1-S 3'UTR into *pTK-luc* vector and then sequenced the clones. After that luciferase assay was performed. To obtain 3'UTR of alternatively spliced ITSN1-22a isoform that contains exon 22a we performed PCR with specific primers and then cloned PCR products into *pTK-luc*. Then luciferase assay was performed. **Results**. Recently using several bioinformatical web-servers we have found potential target sites for microRNAs and AU-rich binding protein tristetraproline (TTP) in 3'UTR of ITSN1-S mRNA. Luciferase assay showed the 10-fold inhibition of luciferase activity of the construct containing 3'UTR of ITSN1-S mRNA in HEK293 cells and 4.7-fold inhibition in HeLa cells compared to the intact *pTK-luc* vector. For further investigation of the affect of potential target sites in 3'UTR on ITSN1-S mRNA expression we constructed 6 deletion mutants based on full-length *pTK-luc*-3'UTR ITSN1-S construct, and luciferase assay using these constructs was performed. We also analyzed the impact of 3'UTR of mRNA of ITSN1-22a isoform on the expression of reporter gene of luciferase. No inhibition of the expression of reporter gene of luciferase by the 3'UTR of mRNA of ITSN1-22a isoform has been shown. On the contrary, different levels of inhibition of luciferase activity of constructs with mutant 3'UTR of ITSN1-S mRNA have been shown. In order to understand if the repression occurs due to microRNAs or other regulatory factors action or to the size of transfected constructs we plan to create «pseudo-3'UTR» construct based on *pTK-luc* vector containing an insertion of the same size as the full-length 3'UTR of ITSN1-S mRNA. While analyzing the results of luciferase assay we have found that one of the deletion mutants shows practically no luciferase activity in HEK293 cells. This mutant is characterized by deletion for TTP target site and target sites for several microRNAs including predicted site for hsa-miR-10a. Recently it has been shown that in some cases human microRNAs from miR-10 family could be positive regulators of gene expression. At the same time we did not observe similar results for other mutants with deletion of the regions with analogous target sites. To understand the nature of such ambiguous data we plan to perform luciferase assay using a new construct based of *pTK-luc* vector with insertion of 500 bp region contained target sites that were deleted in mutant without luciferase activity. **Conclusions**. Using luciferase assay we identified different impacts of the full-length 3'UTR of ITSN1-S mRNA and 6 deletion mutants on the expression of the reporter luciferase gene. The results obtained for one of the mutants showed the absence of expression of this construct in HEK-293 cell line. We plan to perform more detailed analysis of the 500 bp region which deletion leads to the complete repression of luciferase activity.

Biosensors based on multi-walled carbon nanotubes and screen-printed gold electrodes

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Aim. To develop new amperometric biosensors on the base of screen-printed three-electrode transistors C220AT (gold electrodes) («DropSens», Spain) and immobilized oxidoreductases, namely glucose oxidase (GOx) (EC 1.1.3.4), choline oxidase (ChOD) (EC 1.1.3.17) and recombinant yeast methyl amine oxidase (MAO) (1.4.3.6) for determination of glucose, choline and methyl amine, correspondingly. To investigate an effect of multi-walled carbon nanotubes (MWCNT), as a component of bioselective membranes, on the biosensors analytical characteristics. To optimize the composition and analytical characteristics of the biosensors and the measurement conditions for determination of each substrate. **Methods**. The methods used for enzyme immobilization were as follows: cross-linking with BSA in glutaraldehyde (GA) vapour, carbodiimide method, electropolymerization with poly(3,4-etylendioxytiofen). Experiments were carried out by cyclic voltammetric and amperometric methods using the Stat 200 device («DropSens», Spain). The spectrophotometric method described by Haywood & Large (1981) was used for assay of AMO activity. **Results**. The method of formation of the bioselective matrix, based on immobilized oxidoreductases with MWCNT-NH_2, on the surface of gold electrodes was developed and optimized. MWCNTs-NH_2 (2 mg/ml) were suspended in polyvinylpyrrolidone (PVP) aqueous solution (100 mg/ml) by sonication for 30 min. A drop (1.7 μl) of the membrane mixture (0.05 % enzyme, 6 % BSA, 2 % MWCNT-NH_2 suspension, and 5 % glycerol in 25 mM phosphate buffer, pH 7.0) was deposited on the working electrode surface and incubated in GA vapour for 30 min at room temperature. A comparative study on analytical characteristics of the biosensors based on GOx, ChOD, MAO, immobilized by different methods, was performed. The advantages of MWCNT-based biosensors were shown. Optimal working conditions of the biosensors developed were determined. **Conclusions**. MWCNT integration into a bioselective matrix improves the biosensor analytical characteristics, which means: higher signal value, wider linear range of substrate analysis, and possibility of substrate determination in wide range of working potential.

Functional studies of PTEN-FABP4 interaction

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Aim. PTEN is a tumor suppressor with dual protein and lipid-phosphatase activity, which is frequently deleted or mutated in many human advanced cancers. Recent studies have also demonstrated that PTEN is involved in cellular metabolism and lipid turnover regulation, which suggested that PTEN is also a promising target for the development of anti-obesity and diabetes 2 type treatment. We have identified fatty acid binding protein 4 (FABP4) as a PTEN binding partner by the yeast two-hybrid screening and confirmed the interaction by pull-down assays and co-immunoprecipitation of transiently overexpressed and endogenous proteins. At present, we focus on the influence of the functional point mutations of these proteins on the ability of complex formation and their consequent effect on the cell signaling alterations. We chose FABP4 Y19 mutations, which mimic or prevent phosphorylation, and PTEN C124S substitution that blocks its enzymatic activity. **Methods**. To generate functional mutants of FABP4, Y19A and Y19D, we applied site directed mutagenesis on pcDNA3-Myc-FABP4WT construct. Then we verified the generated mutants by DNA sequencing and cloned in pEGFP-C1. The expression of generated constructs in transfected HEK293 cells was confirmed by fluorescent microscopy and WB with aMyc antibody. To find out, whether PTEN activity and FABP4 Y19 phosphorylation affect the interaction, we cotransfected HEK293 cells with a set of wild type and mutant forms of Myc-PTEN (in pcDNA4TO) and GFP-Myc-FABP4. Then we confirmed the expression of both proteins by WB and performed co-immunoprecipitation with aGFP antibodies. The bands of Myc-tagged partners were visualized by WB with aMyc antibodies. To examine, whether ectopic expression of wild type and mutant forms of FABP4 and PTEN modulate PI3K signaling in transfected HEK293 cells, we used pcDNA3-Myc-FABP4WT, -Y19D and -Y19A for cotransfection with pcDNA4TO, pcDNA4TO-Myc-PTEN-WT and -C124S and used anti-phospho S473 PKB/Akt as [an] indicative antibody of PI3K pathway activation. As loading control, the antibody to -actin was used. **Results**. Results of IP suggest that point mutation that inactivates PTEN, as well as FABP4 Y19 mutations does not affect FABP4-PTEN complex formation. It suggests non enzyme-substrate nature of this interaction, the aim of which needs to be further elucidated. Preliminary signaling studies indicated that addition of Y19D mutant form of FABP4 eliminates negative effect of overexpressed wild type PTEN on Akt phosphorylation rate. This seems to mean that FABP4 being phosphorylated by IR kinase subunit can antagonize the PTEN activity directly or via indirect signaling events. The exact mechanism of this action is to be investigated. **Conclusions**. We have characterized FABP4-PTEN interaction by co-immunoprecipitation and cell signaling studies using the set of functional mutants of these proteins, and have found out that FABP4 phosphorylation as well as PTEN phosphatase activity do not affect interaction directly, but may modulate signaling events in PI3K pathway.

Single-chain antibody fusion proteins: design and application in immunoassays

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Monoclonal antibodies conjugated with fluorescent or enzyme tags are valuable highly-specific molecular probes for different kinds of immunoassay. Labeling of antibodies is traditionally achieved by their chemical coupling with a fluorescent or enzyme marker. This is a complicated process, which heightens the cost of the resulting probes. Advanced phage antibody and gene manipulation technologies facilitate production of recombinant antibodies and allow their genetic fusion with other protein partners. Such recombinant proteins can be produced in bacteria by the rapid and cost-effective fermentation process under defined conditions. Thus, the recombinant antibodies genetically fused with marker proteins are an attractive alternative to their chemical conjugates with fluorescent dyes or enzymes. The aim of this work was to generate the genetic fusion of previously obtained single-chain antibodies (scFv) with the red fluorescent protein mCherry and modified bacterial alkaline phosphatase (BAP), as well as to investigate their applicability for different kinds of the immunoassay. The following methods and techniques were used in this work: bacteria cells culturing, gene cloning, polymerase chain reaction, DNA electrophoresis, DNA sequencing, ELISA, Western-blotting, protein expression, protein purification, and protein refolding, fluorescent microscopy. We chose mCherry as a fluorescent fusion partner, because it has high brightness and photostability and is tolerant enough for fusion with other proteins. The genes of the scFv-mCherry fusion proteins were designed and expressed in *Escherichia coli*. The expression yield of the target proteins was optimized and amounted up to 500 mg of scFv-mCherry per 1 l of *E. coli* culture. The fusion proteins have been obtained on multi-milligram scales in a purified and soluble form with rapid and cost-effective on-column refolding process. Immunochemical and immunofluorescent assays confirmed that both moieties of the scFv-mCherry fusion proteins retained their functional activity after the refolding. BAP is a widely used marker protein due to its high sensitivity and stability. Moreover, BAP exists in a dimeric form, thus after the fusion with scFv the avidity of such immunoconjugate redoubles. To obtain an enzyme with enhanced catalytic activity mutagenesis of BAP was carried out. The ScFv-BAP_{mut} fusion proteins were designed and recombinant plasmids for their expression in *E. coli* were created. All fusion proteins were produced in bacteria by secretion into the periplasm and culturing medium. Functional activity for both moieties of the secreted ScFv-BAP_{mut} protein has been shown. Applicability of so engineered alkaline phosphatase immunoconjugates in antigen detection by ELISA, immunoblotting, and immunocytochemistry has been demonstrated.

Highly efficient analysis of cDNA combinatorial libraries of immunoglobulin variable genes for obtaining and characterization of single-chain antibody panel against cell-surface biomarkers on the human CD34 antigen example

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Aim. Development of a universal platform for obtaining and characterization of single-chain Fv-antibodies (ScFv) against cell-surface biomarkers on CD34 antigen example using high-throughput screening assay. **Methods**. mRNA isolation; cDNA synthesis; cloning; phage display that used cell-based schemes of affinity selection; enzyme-linked immunosorbent assay (ELISA); fluorescent ELISA; flow cytometry; DNA sequencing. **Results**. Immune combinatorial library of mouse immunoglobulin *V*-genes of $1.8 10^6$ clones was obtained. A panel of ScFv that recognize antigen on the surface of CD34-positive cell line KG1 was generated. **Conclusions**. A new strategy for obtaining ScFv against cell-surface proteins was proposed. The strategy is based on construction of combinatorial cDNA library of immunoglobulin *V*-genes of animals that were immunized with recombinant antigen, affinity selection of the library using efficient cell-based selection systems and subsequent high-throughput library screening for desired antibodies.

RAPD- and ISSR-analyses of *Gentiana pneumonanthe* L. tissue and organ culture

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The aim of this work was to study the genome variability for *G. pneumonanthe* L. morphogenic and nonmorphogenic cultures *in vitro* by RAPD- and ISSR-PCR. *G. pneumonanthe* specimens from two plant populations, Koryukivs'ke forestry, Chernigiv region, and v. Vygoda, Ivano-Frankivs'k region, were studied. Plants from natural populations, aseptic plants, derived from the seed under aseptic conditions to be used to generate cultures *in vitro* (plant-donors), nonmorphogenic tissue cultures of root origin, embryogenic calli displaying primordial roots and shoots, obtained from the nonmorphogenic tissue cultures at the 5th and 8th passages, as well as isolated root cultures were explored. DNA purification, RAPD- and ISSR-analysis were performed in accordance with the standard techniques. On the basis of the results, obtained through FAMD 1.21 beta program, the dendrograms for *G. pneumonanthe* specimens' genetic likeness in terms of the Jaccard (D_J) genetic distances were constructed. Altogether for RAPD-analysis there were used 27 primers, 15 of which providing the synthesis of clear-cut reproducible amplicons were chosen for the work. For ISSR-analysis 5 primers were used. Total number of amplicons in the case of RAPD-analysis made up 207, 69 of which (33.3 %) were shared by each object. In the case of ISSR-analysis these accounted for 64, 14 of which (21.9 %) were joint. At the dendrograms of the genetic likeness, constructed on the basis of the results of RAPD- and ISSR-analysis, the specimens were divided into two clusters according to the population belonging. The closest to plant-donors were nonmorphogenic tissue cultures at early (5th, 8th, 9th) passages. The Jaccard genetic distances by the results of RAPD-analysis constituted 0.12-0.16, while those of ISSR-analysis were 0.04–0.24. Upon further maintenance the calluses genetic remoteness for RAPD-PCR rose up to 0.17–0.22. The embryogenic cultures in accord with the results were most genetically similar to the calluses from which they were derived (D_J = 0.05–0.09). The isolated root cultures from plants of both populations formed individual branch considerably distinguishing from both original plant (D_J = 0.21) and embryogenic and nonmorphogenic calluses (D_J = 0.15–0.23). The values of the genetic distances between plant-donors and generated from them tissue cultures for RAPD-PCR did not exceed those between the individual remote genotypes of plants from the same natural population of Koryukivs'ke forestry (D_J = 0.26–0.35). RAPD- and ISSR-analyses of *G. pneumonanthe* tissue and organ culture were carried out. The specimen distribution at the dendrograms corresponded to their population belonging. Genome variability for various cultures *in vitro* was found to vary considerably while failing, however, to extend beyond the limits of intrapopulation polymorphism.

New triazinebenzotiazine-6 and 8-carboxylic acids and their amides: synthesis, molecular modeling and biotesting

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Aim. Synthesis of new of 3-oxo-1,2,4-triazino[5,6-b][1,4]benzotiazine (1,2,4-TBT) derivatives, namely, 1,2,4-TBT-6- and 1,2,4-TBT-8-carboxylic acids and their amides; bringing out among them the inhibitors of RNA synthesis and compounds showing an antibacterial effect. **Methods**. Nonempirical quantum-chemical methods for optimization of 1,2,4-TBT structure, molecular docking of ligands into the virtual target, rational chemical synthesis of new substances, their testing in the model transcription system using DNA-dependent RNA polymerase of T7 bacteriophage, and testing on the bacterial culture models. **Results**. Two series of 1,2,4-ТБТ-6- and 1,2,4-ТБТ-8-carboxamides were synthesized. The compounds testing in the transcription system *in vitro* with bacteriophage T7 DNA-dependent RNA polymerase determined their structure- and concentration-dependent inhibition of RNA synthesis. The molecular docking showed a possible behavior of selected ligands in the catalytic pocket of transcription complex. The members of both synthesized series were tested in the bacterial cultures for their ability to repress growth of gram-positive, *Bacillus subtilis, Erysipelothrix rhusiopathiae, Diplococcus lanceolatus, Streptococcus suis* (serotype 1, 2), *Streptococcus lysogenicus* and gram-negative, *Escherichia coli, Klebsiella* spp. bacteria. The examined preparations exhibited differential antibacterial activities. **Conclusions**. The effective inhibitors of the RNA synthesis and the substances displaying the statistically significant antibacterial effect were found among the compounds synthesized. The analysis of the structure-activity relationship of synthesized amides allowed us to delineate further directions for designing new derivatives of 1,2,4-ТБТ-6- and 1,2,4-ТБТ-8-carboxylic acids to improve their biological characteristics.

Investigation and characterization of mTOR-kinase isoforms

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The TOR pathway is a well known conserved pathway from yeast to mammalian cells. The mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase which regulates cell growth and metabolism in response to environmental signals affecting protein synthesis and cytoskeleton dynamics. Deregulation of mTOR-coordinated signaling is associated with various human pathologies, including diabetes, inflammation and cancer. mTOR kinase forms a multisubunit complex with numerous protein partners. In mammalian cells two distinct complexes have been identified: mTORC1 in which mTOR is bound to the protein partner raptor and mTORC2 in which mTOR is bound to another protein partner called rictor. These protein complexes appear to have distinct biological functions. Recently, our research group has identified the existence of potential mTOR splice variants by RT-PCR analysis of various human cell lines. Among them – TOR- isoform, which has been characterized and patented by now. This form shows oncogenic characteristics. So, the aim of our current studies was to prove the existence of mTOR isoform (TOR-) on RNA and protein levels in mammalian tissues and cell cultures. For this purpose different methods of molecular biology were used: molecular cloning techniques; RNA and DNA extraction; Northern blot; Western blot; reaction of immunoprecipitation; stable cell lines techniques. **Results**. The bioinformatical analysis of TOR- and TOR- isoforms primary structures was carried out. It allowed us to reveal the absence of several functional domains in their structure, if compared to a full-length TOR- molecule. Both isoformes TOR- and TOR- were cloned in eukaryotic vector *pcDNA3.1* and stable cell lines were obtained. A fragment of C-terminal part of mTOR was also cloned, overexpressed and purified from bacteria cells. This recombinant protein was used for rabbit immunization and TOR-specific polyclonal antibodies generation. The generated antibodies as well as commercially available antibodies against C-terminal part of mTOR (Millipore) were used for Western blot reaction in a panel of different cell lines, rat tissues and mouse embryo tissues lysates. It allows us to detect in some cell lines and tissues not only 290 kDa protein (TOR-), but also several bands of proteins with lower molecular weight, similar to TOR- , TOR- and TOR- isoforms. **Conclusions**. Several mTOR isoforms were found by RT-PCR technique and cloned. Bioinformatical analysis of the mTOR isoforms structure was performed. Two clones TOR51 and TOR50 were used for the stable cell lines generation. C-terminal fragment of mTOR was cloned, overexpressed and used for TOR-C polyclonal antibodies generation. Further investigations are necessary to prove the existence of alternative forms of mTOR.

Characterization of two members of chitinase-like protein family overexpressed in glioblastoma

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Aim. Chitinase 3-like 1 (CHI3L1, HC gp-39, YKL-40) and chitinase 3-like 2 (CHI3L2, YKL-39) belong to glycosylhydrolase protein family 18 and, as we have found previously, are overexpressed in glioblastoma, the most aggressive type of human brain tumors. However, the expression of these two genes is independent on both mRNA and protein levels. The aim of this investigation is sequence-based prediction of 3D structure of human mature CHI3L2, closely related to CHI3L1, comparison of functional features of these homologous proteins in terms of their 3D structures, and characterization of their functional properties. **Methods**. The crystal structures of homologous CHI3L1 protein in free state (Protein Data Bank codes: 1HJX:A and 1NWR:A) were used as structural templates for CHI3L2 homology modeling. The CHI3L2 model ensemble was constructed using Modeller 9.7 program, and the best model was selected by the smallest value of normalized Discrete Optimized Molecule Energy (DOPE) score. Structures from the model ensemble were assessed by means of PSVS server and MolProbity. The protein coding region of CHI3L1 cDNA sequence was amplified from a plasmid containing CHI3L1 insertion and cloned in *pET-23d*(+) expression vector at restriction sites *NcoI* and *XhoI*. His_6-tagged CHI3L1 was purified on Ni-NTA agarose. Native CHI3L1 was purified from conditioned medium of MG-63 human osteosarcoma cell line by affinity chromatography using heparin-sepharose. Proliferation was measured using MTS assay. Motility was investigated using wound healing assay. **Results**. Oligosaccharide-binding groove of CHI3L1 is lined with aromatic residues, which have hydrophobic stacking interactions with the hydrophobic sides of the bound sugar rings. Trp^{352} is conservative in CHI3L2 ligand-binding groove, but Trp^{99} is replaced by Tyr^{104}. Trp^{69} and Trp^{71} hydrophobic residues of CHI3L1 are replaced in CHI3L2 by positively charged Lys^{74} and Lys^{76}, and both substitutions could strongly change the polarity and ligand-binding specificity of CHI3L2 ligand-binding groove, which potentially binds heparan sulfates. CHI3L1 is N-glycosylated while CHI3L2 is not. We investigated CHI3L1-induced proliferation and motility on several glioma cell lines. CHI3L1 like IGF-1 stimulates proliferation of U373 glioma cells. Wound healing assay revealed CHI3L1 stimulation effect on U87 but not on U251 motility. U87 cell line has features of more malignant cells, it corresponds to WHO astrocytoma grade IV. **Conclusions**. CHI3L2 and CHI3L1 differ in active site hydrophobicity and charge, CHI3L2 has mutated heparin-binding motif thus it does not bind heparin. CHI3L1 possesses proliferation potential and stimulates glioma cell motility. Further characterization of the functional properties of CHI3L1 and CHI3L2 is necessary for elucidation of the role of CHI3L1 and CHI3L2 proteins in glial tumors formation.

Institute of Molecular Biology and Genetics NAS of Ukraine, 2010

Comprehensive conformational analysis of 2',3'-didehydro-2',3'-dideoxy analogues of natural nucleosides as a key to understanding their biological activity

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The aim of this work is to cast a light upon a biological activity of 2',3'-didehydro-2',3'-dideoxy analogues of natural nucleosides, HIV-reverse transcriptase inhibitors (d4T, d4U, d4C), by investigating their conformational space. Conformational search and analysis were performed at the MP2/6-311++G(d,p)//DFT B3LYP/6-31++G(d,p) level of the theory. The study on electronic density topology, based on the QTAIM (quantum theory of atoms in molecules) approach, was carried out for all conformers in order to identify possible hydrogen (H) bonds, which were also tested by geometric and vibrational criteria. The full conformational families of modified nucleosides proved to be rather small (from 15 to 16 conformers within the 4.74 5.51 kcal/mol Gibbs energy range) due to structural rigidity of the sugar moiety. It was shown that global minima on Gibbs energy surface at 298.15 K correspond to the anti conformer. The sugar residue was found to be flattened as compared to natural nucleosides. It is interesting that C2'endo and C3'endo sugar subfamilies are not the most densely populated in contrast to canonical nucleosides. Geometrical, vibrational, structural-topological, and energetic features of intramolecular H-bonds in the calculated conformers were determined. Based on the QTAIM analysis, 8 types of H-bonds, involving bases and sugar, were detected numerically from 1 to 2 per a conformer: C6H...O5', C6H...O4', O5'H...O2, C5'H1'...O2, C5'H2'...O2, C1'H'...O2, C5'H1'...HC6, C5'H2'...HC6. The full conformational families of d4T, d4U and d4C include only one DNA-like conformer (g^+, t, anti). The calculation of «ideal» DNA-like structures with fixed angle corresponding to the average experimental values for A (= 201.1) and B (= 258.1) DNA forms, shows that B-like conformers of all the nucleosides are more stable than A-like ones. The comprehensive conformational analysis of d4T, d4U, d4C nucleosides as potential reverse transcriptase inhibitors was performed for the first time. The similarity of A and B DNA-like conformers of the modified and corresponding natural nucleosides leads us to think that 2',3'-didehydro-2',3'-dideoxy analogues most likely act as polynucleotide chain terminators which is due to the absence of the hydroxyl group at 2' position of the sugar ring.

Investigation of N*KIRAS1* gene alteration in human renal cell carcinomas

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Renal cell carcinoma (RCC) is the most common malignant kidney tumor in adults. We chose the most predominant type, clear cell carcinoma, for this study and performed a large-scale analysis of gene expression profile. The identification of genetic/epigenetic changes and gene expression pattern in RCC might lead to the identification of the set of putative disease markers. Using *NotI* microarrays we confirmed that *NKIRAS1* (NF- B inhibitor interacting Ras-like 1 gene) showed the highest degree of genetic/epigenetic changes. The NF- B transcription factor regulates cell proliferation, apoptosis mediated by tumor necrosis factor (TNF), expression of genes responsible for both innate and adoptive immune response. It is also implicated in synaptic plasticity and memory. Thus, 78 % of the cases demonstrated hemizygous deletion/methylation in the *NKIRAS1* gene locus. To validate the results of NotI microarray analysis we studied the expression level and copy number of *NKIRAS1* gene. In our work we used the frozen material of a surgically removed tumor and surrounding normal tissues. For global scanning of chromosome 3p we used *NotI* microarray. Bisulfite sequencing was used for studing DNA methylation of *NKIRAS1*. Q-PCR was performed, using SYBR Green Mastermix (Fermentas) and TBP gene as a reference. Copy numbers of *NKIRAS1* gene and its expression level were estimated by the 2- CP method of relative quantification. An analysis of *NKIRAS1* gene expression was performed in 12 tumor samples (1 benign oncocytoma, 1 papillary cancer and 10 of RCC) by Q-PCR. *NKIRAS1* expression was down-regulated in 73 % of RCC samples (8 of 11) as compared to surrounding normal tissue. High grade tumors (3 and 4) showed lower expression of *NKIRAS1* at the mRNA level than tumors of low grade (1 and 2). No significant association was found between gene expression level and gender or age. Copy number of the *NKIRAS1* gene was analyzed in 19 tumor samples (3 benign oncocytomas, 1 papillary cancer, 1 sarcoma and 14 RCC). Changes in the copy number of *NKIRAS1* gene were observed in 64 % (9 of 14) of RCC samples. 9 samples displayed the ratio < 0.85 and > 0.5, thus they were considered as hemizygous deletions. 5 samples showed the ratio > 0.85 and were considered as normal. Changes were found in all 3 benign oncocytomas, 1 papillary cancer and 1 sarcoma, where hemizygous deletion was observed. We describe a novel member of RAS family which is associated with tumor growth and metastasis in epithelial cancers. Copy number of *NKIRAS1* gene decreases in renal cell carcinomas and its expression decreases in malignancies. We plan to investigate the association of *NKIRAS1* gene with age and a possibility to use it as a diagnostic marker for renal cell carcinoma. We also plan to investigate a role of NKIRAS1 protein in the development of renal cancer.

Functional role of PH and C2 domains of Bcr/Abl protein in development of Ph-positive leukemias

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Introduction. Hybrid protein Bcr/Abl, a product of chromosomal translocation t(9;22), is believed to be a key factor of malignant transformation in Ph-positive leukemias. It is important to clarify a functional role of domains of this protein, which is realized through an interaction with other molecules. In previous studies, Hsp27 and cortactin were identified as likely candidates for binding PH domain of Bcr/Abl. Hsp27 is of interest as a possible regulator of IKKb activation. Cortactin is involved in receptor internalization and endocytosis. The structure and function of C2 domain have not yet been investigated. **Aim**. Development of the recombinant construction for expression of the protein similar to C2 domain of Bcr/Abl protein for further studies of its function. Generation of recombinant proteins (Hsp27 and cortactin) to prove the interaction with Bcr/Abl protein PH domain. **Methods**. The recombinant constructions were developed using genetic engineering methods (design of specific olgonucleotide primers, PCR, cloning of amplification fragment into expression vector, verification of obtained constructions). Recombinant C2 domain of Bcr/Abl was purified with Ni-NTA agarose. **Results**. *pET28a/C2* construction was developed and verified. Recombinant C2 domain was successfully expressed and purified. Recombinant constructions that encode Hsp27 and cortactin were developed on the basis of *pGEX-4T*. **Conclusions**. The construction *pET28a/C2* (in *Escherichia coli* strain BL21(DE3)) was effective for gaining the recombinant C2 domain. The recombinant constructions will be used for future analysis of their interaction and role in signaling pathways during development of Ph^+ leukemias.

On-column refolding of recombinant proteins from Escherichia coli inclusion bodies

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Recombinant DNA technology allows cloning and synthesis of target genes in a foreign cell. Today, *Escherichia coli* is the commonest expression system for a wide variety of scientifically and commercially impor tant recombinant proteins. The advantage of such synthesis is its high level of accumulation and purity of the recombinant proteins stored as insoluble cytoplasmatic aggregates – inclusion bodies. One of the common problems here is the necessity of solubilization and renaturation of the recombinant proteins from bacterial inclusion bodies. Recombinant protein refolding is an empiric process and requires specific protocols for any particular protein. Binding of denatured proteins from inclusion bodies through a His-tag to Ni-NTA resin is also used for one-step on-column purification and renaturation of recombinant proteins. Protein renaturation is achieved by means of controlled decrease of the concentration of denaturing reagent and can be optimized by varying the renaturating buffer composition and the chromatographic process parameters. **Aim**. The aim of the present work was to optimize the renaturation scheme for different recombinant proteins using immobilizing metal affinity chromatography (IMAX) and determine functional characteristics of these proteins. **Methods**. The following methods and techniques were used: bacterial cell culture, protein expression, isolation of inclusion bodies, protein purification, protein refolding, protein electrophoresis, ELISA, Western-blotting, Ni_2^+ chelate affinity chromatography, SEC-chromatography. **Results**. The expression of different recombinant proteins was optimized in *E. coli*. The effective schemes of their obtaining in a purified and soluble form from *E. coli* inclusion bodies by renaturation on metal-affinity matrix were developed. For these recombinant proteins, the content of soluble aggregates, efficiency of disulfide bond forming, functional activity, and stability at storage were determined. **Conclusions**. The approach proposed can be used for obtaining biologically active His-tagged proteins consisting, in most cases, of several domains and having disulphide bonds.

Conductometric chemosensors based on calixarenes for determination of amines and amino acids

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Amines are toxic compounds found in oil, food and pharmaceutical industries, as well as in dye manufacturing and agriculture. The need to determine amines precisely has increased over the last few years because of growing environmental problems in agriculture, where amines are used in fertilizers, as well as associated with waste water, where amines are originated from surfactants. Calixarene derivatives, cyclic oligomers of phenolformaldehyde condensates, have been considered as a new type of host compounds since their tetramer, hexamer, heptamer or octamer became easily available from tert-butylphenol and formaldehyde. They form reversible complexes but their host-guest properties can be largely varied due to chemical modification of their basic structure in order to provide the recognition of hydrophilic moieties such as metal ions and small organic molecules. Various calixarene derivatives have been synthesized to interact with amino compounds. Thin-film interdigitated planar electrodes with calixarenes have been used for the development of conductometric amine and amino acid chemosensors. The optimal values of pH, buffer capacity and ionic strength have been determined. Operational stability, storage stability and selectivity of the developed conductometric biosensor have also been analyzed. The optimum pH values for amine sorption were observed at pH = 5.5 for the calixarene derivatives. The experimental results show that C-benzylresorcinolcalixarene exhibited better affinity toward amine species than other macrocycles. This preliminary study has revealed a possibility of using calixarenic doped membranes for determination of pollutants in the solid-liquid interface. This work has demonstrated, i9n particular, the use of C-benzylresorcinolcalixarene, *p*-tert-butylcalix[4]arene and *p*-tert-butylcalix[8]arene as receptors of selective conductometric chemosensors for detection of organic amines and amino acids in aqueous phase. The sample solution was found to have effect on the sensor sensitivity owing to the changes in the chemical properties of the immobilization mixture. Strong interactions, such as acid–base ones, and complexation between analytes and calixarene increased the sensor response in buffer solution. The method was quite reproducible and the sensor responses were stable in time.

Secondary structure of 3' end of HIV-1 pre-mRNA 3' untranslated region

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Aim. Both 5' and 3' untranslated regions of HIV-1 transcript contain a set of structural elements directing certain stages of viral replication. Though many structural models of HIV-1 pre-mRNA 5' UTR are reported in literature, the secondary structure of 3 UTR has not been presented until now, except for TAR and polyA hairpins located at both ends of transcript. Among elements in 3' UTR are those essential for 3' processing of HIV-1 transcript including functional poly(A) site. Unique feature of HIV-1 genome is its high heterogeneity, which significantly impedes AIDS diagnostics, however on the other hand this feature enables to use phylogenetic approach to study mechanisms of the virus replication. The objective of this study was to conduct phylogenetic analysis and secondary structure prediction of 3' end of HIV-1 pre-mRNA 3' UTR extended from polyA hairpin up to the end of the transcript. **Methods**. The HIV-1 genomic sequences have been extracted from GenBank. The secondary structure of this region has been predicted by mfold program of M. Zuker. **Results**. Totally we have analyzed 400 HIV-1 genomic sequences and observed two alternative structures of 3 end of HIV- 1 pre-mRNA 3' UTR, depending on HIV-1 subtype. In all HIV-1 isolates of B or C subtype, many iso- lates of D subtype and also some isolates of other subtypes, and recombinant forms the region under study is folded into three distinct hairpins, namely polyA hairpin, a hairpin with poly(A) site down stream element (DSE), exposed in its apical loop (we named it DSE hairpin), and U5(U) hairpin. The U5(U) hairpin is commonly formed upon PBS domain folding and we suppose that alongside with its function in reverse transcription it can play an additional role in polyadenylation process providing an exposure of GU/U tract to CstF during transcription of proviral DNA. In most HIV-1 isolates of E (A/E) or G subtype, PBS domain of which contains U5(AA) hairpin instead of U5(U) hairpin, the region under study is also folded with formation of three distinct hairpins, namely polyA hairpin, a long hair pin with DSE exposed in its internal loop and an upper part of U5(AA) hairpin. The latter structure is also formed in some isolates of D and A subtypes and some recombinant forms. **Conclusions**. Thus, DSE is completely exposed to cellular polyadenylation machinery during transcription of 3' end of 3' LTR HIV-1 proviral DNA that contributes to effective polyadenylation of HIV-1 transcript at 3' poly(A) site.