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MALDI MASS SPECTROMETRY OF ZIRCONIUM AND HAFNIUM DIBENZOYLMETHANATE PHTHALOCYANINES *

The GALDI mass spectrometry studies have shown the noticeable distinction in behavior of similar structure phthalocyanine complexes of zirconium and hafnium containing dibenzoylmethane groups as out-of-plane ligands. The hafnium phthalocyanine $PcHfDbm_2$ is an association prone and form different types of associates ($[(PcHfDbm)_2]^+$, $[PcHfDbm+PcHfDbm_2]^+$) in positive ion mode and ($[(PcHfDbm_2)_2]^-$) in negative ion mode, while for zirconium phthalocyanine $PcZrDbm_2$ no associates were observed. Furthermore two pathways of fragmentation are suggested for these complexes. The first pathway occurs for both $PcZrDbm_2$ and $PcHfDbm_2$ in positive and negative ion modes, while second one only for $PcHfDbm_2$ in negative ion mode. Interaction of phthalocyanines with fibrillogenic protein insulin was studied by MALDI mass spectrometry. In presence of $PcZrDbm_2$ and $PcHfDbm_2$ the disappearance of peaks of low-molecular aggregates of insulin is observed, that points at interaction of phthalocyanines with protein early aggregates.

INTRODUCTION. Due to the unique optical, semiconductor and photochemical properties phthalocyanine macrocycles have a good prospects for application in various fields of science and technology and thus are extensively studied [1]. Generally for the modification of the structure and hence properties of phthalocyanine systems the imposition of central metal atom or substituents on the periphery of the macrocycle is used [2—4]. In recent years the approach of modification of phthalocyanine through the incorporation of out-of-plane ligands is widely developed. Presence of ligands in this position noticeably affects the π-electron conjugated system of macrocycle that changes electron structure of macrocycle and its spatial characteristics.

The phthalocyanines $PcZrDbm_2$ and $PcHfDbm_2$ containing dibenzoylmethane as out-of-plane ligand were firstly synthesized and characterized using several physico-chemical methods. The data of X-ray analysis, ^{1}H NMR spectroscopy and absorption electronic spectroscopy demonstrated the very similarity of molecule spatial structures and characteristics of zirconium and hafnium phthalocyanines [5]. At the same time the GALDI (graphite activated laser desorbtion/ionization) mass spectrometry method has shown the noticeable difference in behavior of these complexes. The compound $PcHfDbm_2$ has the pronounced tendency to association [5] in the gas phase and formation of high molecular weight products, the same tendency to association was also observed for some other phthalocyanines [6]. In opposite, in spectra of $PcZrDbm_2$ the peaks of ions with masses higher than molecular mass are not observed. This distinction between the behavior of Zr and Hf phthalocyanines in the gas phase was firstly reported in [5]. The detailed studies of this phenomenon is considered to be of interest since due to the very close chemical properties of Zr and Hf, their compounds usually possess the similar properties and behavior.

The electro- and photocatalytic, electrochromic properties and biological activity of zirconium and hafnium phthalocyanines containing out-of-plane ligands were reported previously [7, 8]. Recently it was shown that phthalocyanines with out-of-plane ligands are able to redirect the reaction of insulin fibrillization and this way to prevent the formation of amyloid fibrils. The $PcZrDbm_2$ was reported among the compounds possessing high anti-fibrillogenic properties [9].

In the current paper the detailed studies of $PcZrDbm_2$ and $PcHfDbm_2$ phthalocyanines by the GALDI mass spectrometry method and the supposed mechanisms of fragmentation of macrocycles are reported. To clarify the mechanism of anti-fibrillogenic activity of phthalocyanines with out-of-

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plane ligands the interaction of $PcZrDbm_2$ and $PcHfDbm_2$ with fibrillogenic protein insulin was characterized using MALDI mass spectrometry method.

EXPERIMENTAL PROCEDURE. Phthalocyanine complexes $PcZrDbm_2$ and $PcHfDbm_2$ were obtained by exchange reaction between dibenzoylmethane and phthalocyanines of zirconium and hafnium dichlorides. Synthesis, structure and properties of these complexes are described in the article [5].

Mass-spectroscopic studies were carried out on Autoflex II (Bruker Daltonics, Germany) mass spectrometer, with nitrogen laser (λ = 337 nm). Spectra were obtained by GALDI method for free $PcZrDbm_2$ and $PcHfDbm_2$ and by MALDI method for insulin — phthalocyanine mixture. Spectra were recorded using different registration modes (positive/negative, linear/reflectrone) and processed using mMass program [10].

Solutions of phthalocyanines and insulin were prepared according to the methodic used in studies of anti-fibrillogenic activity of insulin [9]. Stock solutions of phthalocyanines $PcZrDbm_2$ and $PcHfDbm_2$ of 0.01 mg/ml concentration were prepared in THF. Stock solution of bovine insulin (Sigma-Aldrich) of 1 mg/ml concentration was prepared in 0.1 M HCl.

The working solutions were obtained by mixing the phthalocyanines and insulin solutions in 1:1 volume ratio. As a matrix for studies of insulin-containing samples the saturated solution of sinapic acid (Sigma-Aldrich) was used. The matrix and working solution were mixed in 1:1 ratio. The samples of 4 μl volume were put to standard steel target.

RESULTS AND DISCUSSION. Mass spectrometry studies of $PcZrDbm_2$ and $PcHfDbm_2$. For amplifying of the ionization the phthalocyanines $PcZrDbm_2$ and $PcHfDbm_2$ were characterized by GALDI method. In positive and negative linear modes except the peaks of molecular ions and products of fragmentation or association the wide range of side signals was observed (table). Thus for the increasing of spectra resolution and removing of the peaks of short-living components the reflectron mode was used. The masses of main fragments of phthalocyanines are presented in the table.

Mass of main ions $PcZrL_2$ and $PcHfL_2$ (Da), **detected on different registration modes** (L = Dbm)

Fragments	Nominal ion mass, Da							
	LP+		LP–		RP+		RP–	
	Zr	Hf	Zr	Hf	Zr	Hf	Zr	Hf
[Dbm]			223.3	223.6			223.0	223.1
Fragmentation								
[PcMO]	619.3	708.0	619.2	708.9			617.9	707.7
[PcML]	825.8	915.2	826.6	914.4	824.7	915.0	824.8	914.9
[PcML+K]	862.0	954.0			864.1	954.5		
$[PcML_2]$	1048.7	1138.3	1049.8	1137.8	1047.6	1138.0	1047.7	1137.7
Association								
$[(PcML)_2]$		1828.8				1826.7		
$[(PcML)_2+K]$		1868.0				1869.2		
$[PcML+PcML_2]$	1875.0	2051.0				2051.7		
$[(PcML_2)_2]$		2276.5		2278.5				2275.0

POSITIVE REFLECTRON MODE. In the positive ion mode the spectra of $PcZrDbm_2$ and $PcHfDbm_2$ are similar in the range below molecular ion mass. In this region, peaks of molecular ions 1047.6 Da $[PcZrDbm_2]^+$ and 1138.0 Da $[PcHfDbm_2]^+$ were observed (fig. 1, peaks *3*, *3**); as well as the peaks of highest intensity that belong to fragments containing one Dbm ligand 824.7 Da $[PcZrDbm]^+$ and 915.0 Da $[PcHfDbm]^+$ (fig. 1, peaks *1*, *1**); and less intensive peaks of their potassium adducts 864.1 Da $[PcZrDbm+K]^+$ and 954.5 Da $[PcHfDbm+K]^+$ (peaks 2, 2*). The higher intensity of peaks of fragments $[PcMDbm]^+$ and their ability to form potassium adducts, allow suggesting the higher stability of $[PcMDbm]^+$ comparing with molecular ions $[PcMDbm_2]^+$.

The noticeable difference between spectra of $PcZrDbm_2$ and $PcHfDbm_2$ exists in the region exceeding the molecular ion mass. In spectrum of phthalocyanine $PcHfDbm_2$ the peaks of dimer $[(PcHfDbm)_2]^+$ (1826.7 Da, fig. 1, peak *4**), its potassium adduct $[(PcHfDbm)_2+K]^+$ (1869.2 Da, fig. 1, peak *5**), and $[PcHfDbm+PcHfDbm_2]^+$ associate (2051.7 Da,

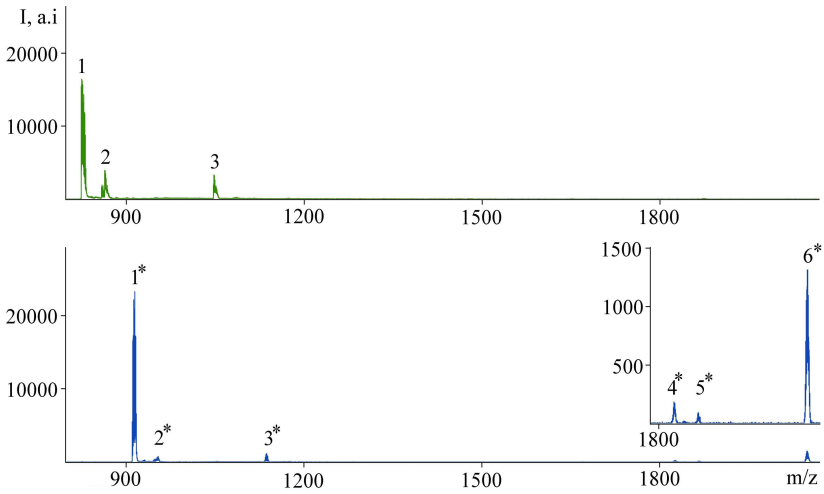

Fig. 1. Mass spectra of $PcZrDbm_2$ and $PcHfDbm_2$ in positive ion reflectron registration mode (*1–3* — signals that correspond to $PcZrDbm_2$; *1*–6** — signals that correspond to $PcHfDbm_2$).

fig. 1; peak *6**) are present. At the same time, for the $PcZrDbm_2$ the peaks of association products are not observed (table). This indicates the pronounced ability of hafnium phthalocyanine to association in gas phase comparing with its zirconium analogue. Supposed mechanism of fragmentation of $PcZrDbm_2$ and $PcHfDbm_2$ and mechanism of $PcHfDbm_2$ association are presented at fig. 2.

NEGATIVE ION REFLECTRON MODE. The spectra of $PcZrDbm_2$ and $PcHfDbm_2$ in negative ion mode have more complicate character comparing with that in positive mode (fig. 3). In spectra besides the peaks of molecular ions $[PcMDbm_2]^+$ (1047.7 and 1137.7 Da for Zr and Hf complexes; peaks *4*, *4**) and fragmentation products $[PcMDbm]^+$ (824.8 and 914.9 Da for Zr and Hf complexes; peaks *3*, *3**) also the peaks of fragments $[PcMO]^-$ (617.9 and 707.7 Da for Zr and Hf complexes; peaks *2*, *2**) and ligand $[Dbm]^-$(223.0 Da; peaks *1*, *1**) are observed (table). Except this, the noise peaks and peaks of associates of fragments with water are present in spectra. Morphology of the peaks points on metal ion content in these fragments.

Instead of associate peaks $[(PcHfDbm)_2]$ and $[PcHfDbm+PcHfDbm_2]$ observed in positive ion mode spectrum of $PcHfDbm_2$, in negative mode spectrum only peak of the molecular ion dimer $[(PcHfDbm_2)_2]^-$ (2275.0 Da; fig. 3, peak *5**) was detected (table).

The spectrum of $PcHfDbm_2$ contains the peaks of oxo form fragments $[PcHfDbmO_2]^-$, $[PcHfDbmO]^+$ and $[PcHfO_2]^-$ with mass 946.8, 930.3, 722.9 Da correspondingly (fig. 4). Formation of fragments of same composition is not detected for $PcZrDbm_2$. As it was mentioned above, in low molecular region of spectra of both phthalocyanines the peak of ligand $[Dbm]^-$ (223.0 Da) is observed.

Basing on the presented data the two path-

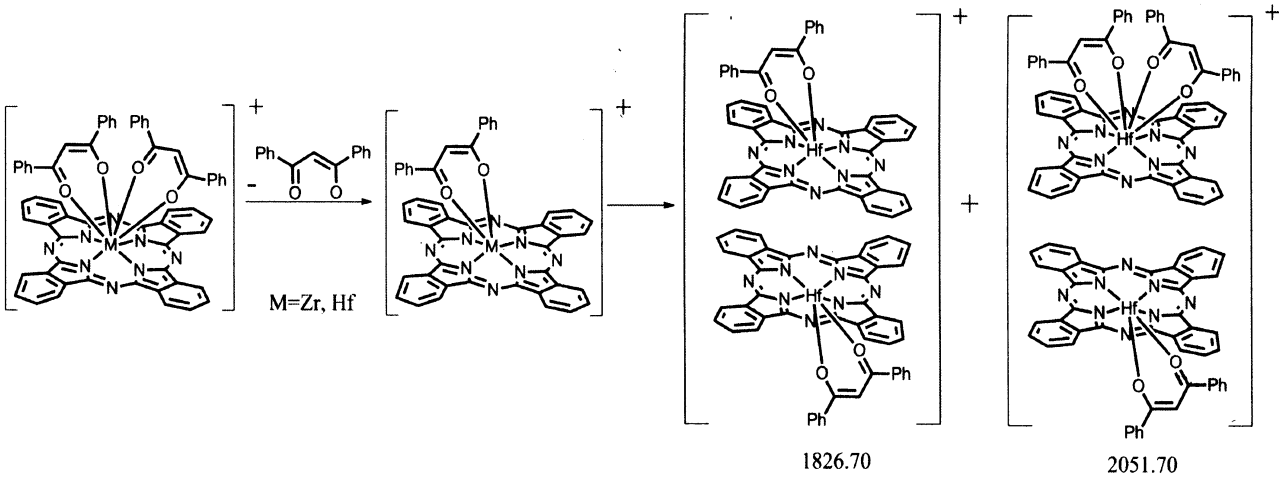

Fig. 2. Supposed mechanism of fragmentation for both phthalocyanines ($PcMDbm_2$ and further association of $PcHfDbm_2$ in positive ion reflectron registration mode.

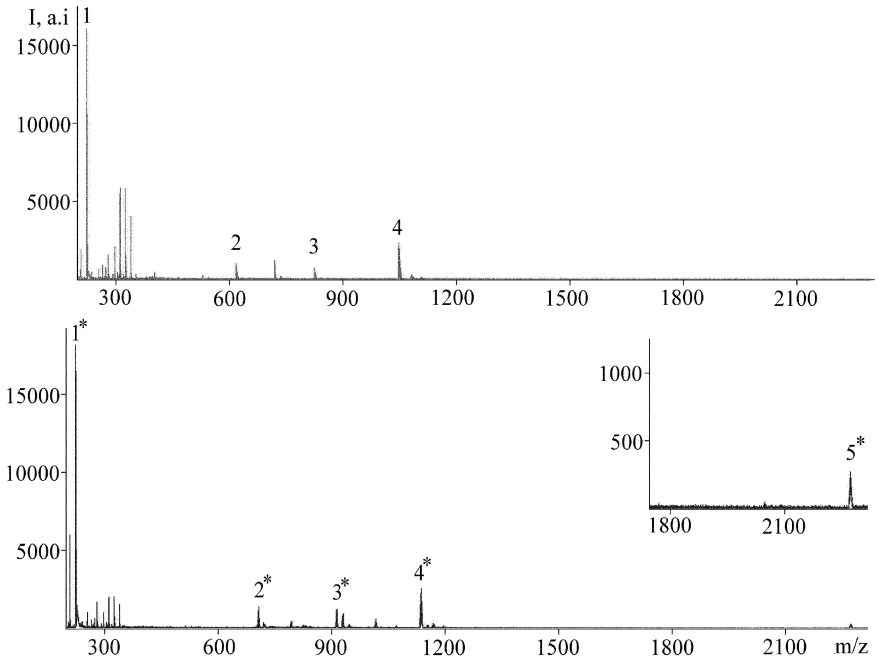

Fig. 3. Mass spectra of $PcZrDbm_2$ and $PcHfDbm_2$ in negative ion reflectron registration mode (*1–4* — signals that correspond to $PcZrDbm_2$; *1*–5** — signals that correspond to $PcHfDbm_2$).

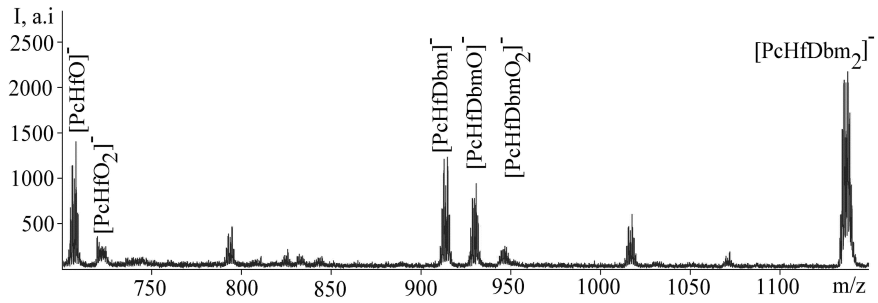

Fig. 4. The region of mass 700—1150 of $PcHfDbm_2$ spectrum in negative ion reflectron registration mode.

ways of fragmentation of phthalocyanines could be suggested. The first mechanism is the elimination of out-of-plane ligand Dbm without it destruction (fig. 5, pathway 1). The second mechanism is the elimination of fragment of out-of-plane ligand, in this case the oxygen atoms persist bound to hafnium atom (fig. 5, pathway *2*). The first fragmentation mechanism is observed for both phthalocyanines in positive (fig. 2) and negative ion modes (fig. 5, pathway *1*), while the second mechanism occurs only for $PcHfDbm_2$ in negative registration mode (fig. 5, pathway *2*).

MALDI MASS SPECTROMETRY STUDIES OF INSULIN IN THE PRESENCE $PcZrDbm_2$ AND $PcHfDbm_2$ IN POSITIVE ION REFLECTRON MODE. For current studies, the insulin and its complexes with $PcZrDbm_2$ and $PcHfDbm_2$ are prepared in acidic medium (0.1 M HCl, pH 1.8) according to methodic used for fibrillization reaction. At low pH the insulin molecules transit to partially unfolded conformation, which is prone to association. This is required for the start of protein aggregation and passing of fibrillization reaction [11]. The MALDI mass spectrum of insulin in positive ion mode contains peak of molecular ion about 5730 Da, and range of decreasing intensity peaks of insulin aggregates: dimer, trimer and tetramer about 11460, 17190 and 22920 Da correspondingly (fig. 6, top). The formation of range of oligomers by insulin at pH 2 was earlier confirmed by nano-flow electro spray mass spectrometry [11].

In spectra of phthalocyanine — insulin mixtures only intensive peaks of molecular ions of individual insulin and $PcZrDbm_2$ or $PcHfDbm_2$ are observed (fig. 6, bottom). The peaks of insulin-$PcMDbm_2$ complexes are not detected neither in linear non in reflectron mode. It could be concluded that insulin with phthalocyanines do not form complexes stable upon mass spectrometry experiment.

However, in presence of phthalocyanines the noticeable changes in mass spectra of insulin occur. The addition of $PcZrDbm_2$ or $PcHfDbm_2$ cause the disappearance of peaks of low-molecular protein aggregates (dimer, trimer and tetramer). It is supposed that phthalocyanine molecules interact with early aggregates causing either their degradation to monomeric insulin or promote the further protein association and formation of high weight insulin

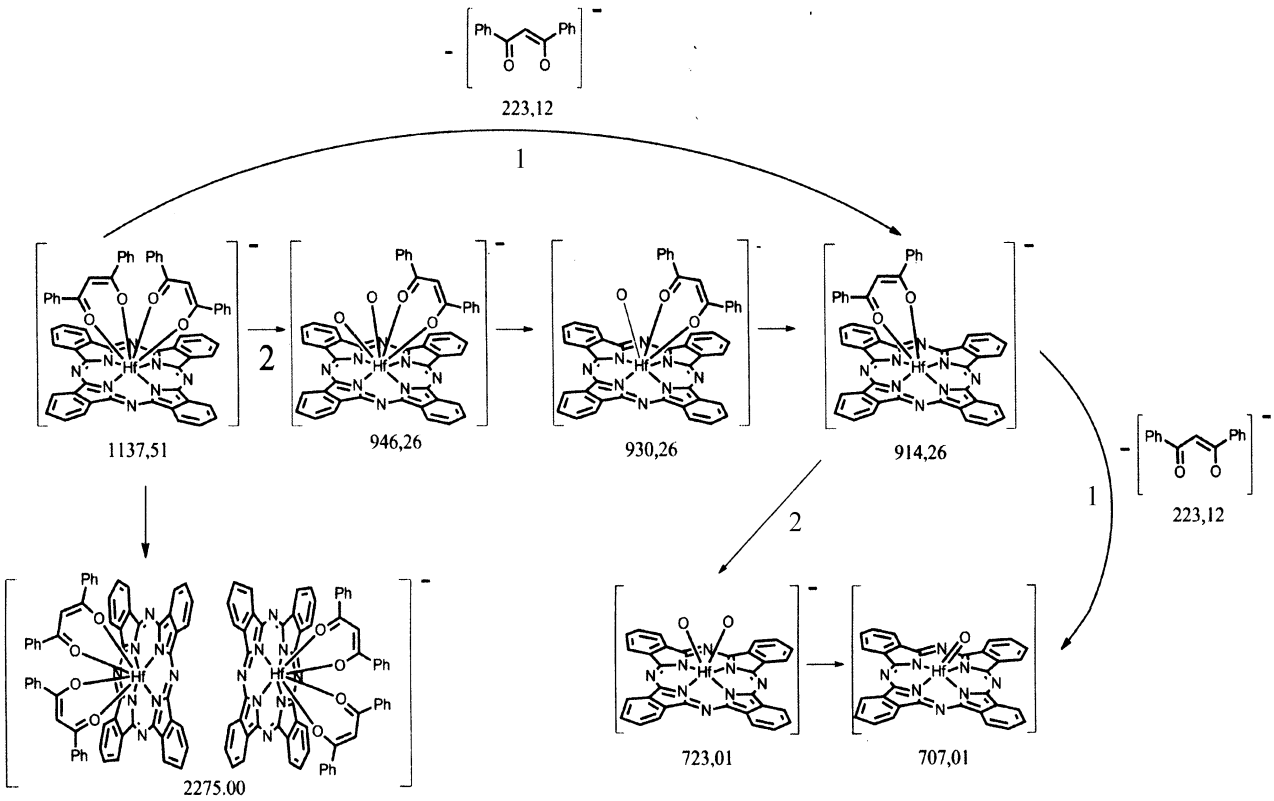

Fig. 5. Mechanisms of fragmentation of $PcHfDbm_2$ that occur in negative ion reflectron registration mode (*1* — elimination of out-of-plane ligand, *2* — destruction of out-of-plane ligand).

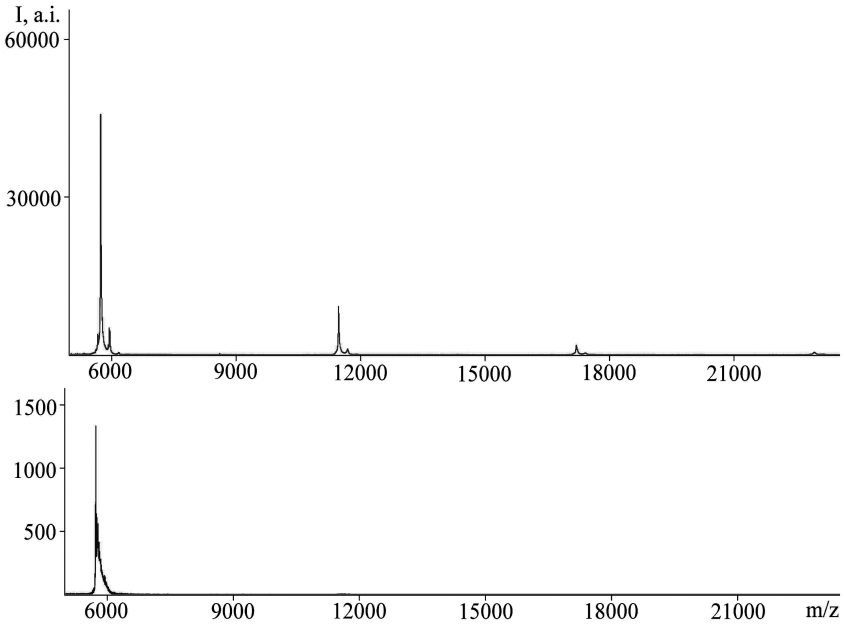

Fig. 6. Mass spectra of insulin (top) and insulin in presence of $PcHfDbm_2$ (bottom).

aggregates that are not detectable by MALDI method.

The interaction of phthalocyanines containing out-of plane ligands with insulin during the passing of fibrillization process is under investigation now.

CONCLUSIONS. The studies by GALDI mass spectrometry method have shown that $PcHfDbm_2$ is association prone and forms different types of associates both in positive ($[(PcHfDbm)_2]^+$, $[PcHfDbm+PcHfDbm_2]^+$) and negative ($[(PcHfDbm_2)_2]^-$) ion modes, while for phthalocyanine $PcZrDbm_2$ the formation of associates is not observed.

Two pathways of fragmentation of the phthalocyanines are suggested. The first one is the elimination of out-of-plane ligand

Dbm without its destruction, that is observed for both $PcZrDbm_2$ and $PcHfDbm_2$. The second one is the destruction of Dbm ligand with its partial elimination that occurs only for $PcHfDbm_2$ in negative ion mode.

The presence of $PcZrDbm_2$ and $PcHfDbm_2$ causes the disappearance of peaks of low-molecular associates of insulin, existing in the MALDI spectra of free protein. That points on ability of phthalocyanines to interact with insulin early aggregates. The formation of stable upon ionization in gas phase phthalocyanine complexes with monomer or aggregated insulin is not detected.

РЕЗЮМЕ. GALDI масс-спектрометрическими исследованиями показана существенная разница в поведении имеющих подобную структуру фталоцианиновых комплексов циркония и гафния с дибензоилметанатными внеплоскостными лигандами. Фталоцианин гафния $PcHfDbm_2$ имеет склонность к ассоциации и образует разные типы ассоциатов ($[(PcHfDbm)_2]^+$, $[PcHfDbm + PcHfDbm_2]^+$) в режиме регистрации положительных ионов и ($[(PcHfDbm_2)_2]^-$) — в режиме регистрации отрицательных ионов, а для фталоцианина циркония $PcZrDbm_2$ ассоциаты не наблюдались. Предложены два пути фрагментации этих комплексов. Первый путь характерен для $PcZrDbm_2$ и $PcHfDbm_2$ в режиме регистрации положительных и отрицательных ионов, тогда как второй — только для $PcHfDbm_2$ в режиме регистрации отрицательных ионов. MALDI масс-спектрометрией изучено взаимодействие фталоцианинов с фибриллогенным белком инсулином. В присутствии $PcZrDbm_2$ и $PcHfDbm_2$ наблюдается исчезновение пика низкомолекулярных агрегатов инсулина, что свидетельствует о взаимодействии фталоцианинов с первоначальными агрегатами белков.

РЕЗЮМЕ. GALDI мас-спектрометричними дослідженнями показано суттєву різницю в поведінці фталоціанінових комплексів цирконію та гафнію з дибензоїлметанатними позаплощинними лігандами, що мають подібну структуру. Фталоціанін гафнію $PcHfDbm_2$ має схильність до асоціації і утворює різні типи асоціатів ($[(PcHfDbm)_2]^+$, $[PcHfDbm + PcHfDbm_2]^+$) у режимі реєстрації позитивних іонів і ($[(PcHfDbm_2)_2]^-$) — у режимі реєстрації негативних іонів, а для фталоціаніну цирконію $PcZrDbm_2$ асоціати не спостерігалися. Запропоновано два шляхи фрагментації для цих комплексів. Перший шлях має місце для $PcZrDbm_2$ і $PcHfDbm_2$ у режимі реєстрації позитивних і негативних іонів, в той час як другий — тільки для $PcHfDbm_2$ у режимі реєстрації негативних іонів. MALDI масспектрометрією було вивчено взаємодію фталоціанінів з фібрилогенним білком інсуліном. У присутності $PcZrDbm_2$ і $PcHfDbm_2$ спостерігається зникнення піку низькомолекулярних агрегатів інсуліну, що вказує на взаємодію фталоціанінів з початковими агрегатами білків.

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