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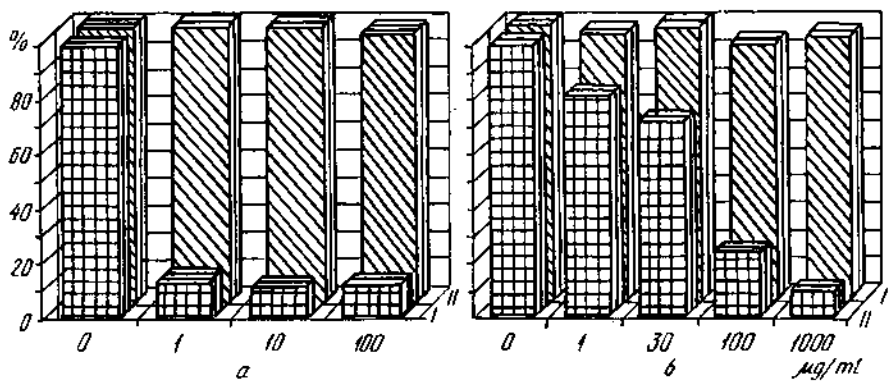
INDIRECT DETERMINATION OF THE 5′→3′ AND 3′→5′ OF THE DNA-DEPENDENT DNA-POLYMERASE FUNCTION

Doxorubicin in the concentration of 1μg/ml and streptonigrin in the concentration 1000 μg/ml each agent separately should by useful to determination of replicative (5′→3′) or reparative (3′→5′) function DNA-polymerase. This enzymes with 5′→3′ fully inhibited by doxorubicine but net sensitive to action of streptonigrin in concentration substances mentioned above. And contraverse, DNA-polymerase with 3′→5′ function don't sensitive to doxorubicin and it's activity depressed by streptonigrin.

Doxorubicin (Adriamycin; $C_{27}H_{29}NO_{11}\cdot HCl$, mm 580.0) and streptonigrin (Nigrin; Bruneomycin; $C_{25}H_{22}N_4O_8$, mm 506.5) (both produced by SIGMA Company, U. S. A) that are inhibitors of nucleic acid synthesis, despite the long history of the studies on them, up to know are considered to be the agents with yet undefined action mechanism and undetermined target for them in prokaryotic cells [1, 2].

During studies on these antibiotics in standard synthesis system of DNA *in vitro* [3], using different prokaryotic DNA-dependent DNA-polymerases with known function (5′→3′ or 3′→5′) and denaturated (single-stranded) DNA as template obtained by either from microorganisms or eukaryotes we have determined that both these agents would had pre-



The dynamic of influence (inhibition percentage) depending on concentration (μg/ml) of doxorubicin (*a*) and streptonigrin (*b*) on the synthesis of DNA *in vitro* processed by DNA-polymerases with 5′→3′ (*I*) or with 3′→5′ (*II*) function

cisely defined targets in a cell of microorganisms and strongly determined and different mechanism action on DNA-polymerases with 5 →3′ or 3′→5′ function.

Doxorubicin practically completely inhibited the activity of the DNA-polymerase with 5′→3′ function (replicase) in the concentration substances in assay about 1 μg/ml and has no effect in the concentration up to 100 μg/ml on the activity of the DNA-polymerase possessing 3′→5′ function (reparase) (figure, *a*).

Streptonigrin also proved to be suitable for differentiation of the forms of DNA-polymerases and determination of their functions: the

forms of DNA-polymerase with 5′→3′ functions were not sensitive to this antibiotic in the concentration of 1000 μg/ml, while the activity of the forms of DNA-polymerases with 3′→5′ exonuclease function was fully inhibited by this concentration of the antibiotics in the assay (figure, *b*).

Streptonigrin is known to cause the formation of single raptures in native DNA in the prokaryotic and animal cells [2]. From our experiment, using single-stranded DNA as template is followed, that this agent doesn't inhibit 3′→5 exonuclease function of DNA-polymerases with reparative function, but it does block reparative centre of this enzyme, what results in fragmentation of template DNA.

Thus, DNA-dependent DNA-polymerases, while realizing replication of genome DNA (replicase) in prokaryoting cells, are the target for doxorubicin, and DNA-dependent DNA-polymerases with 3′→5′ exonuclease and reparative function while realizing reparation of DNA (reparase), are the target for streptonigrin. These antibiotics may be useful in molecular biology (each antibiotic separately in the concentrations mentioned above) as reactive for chemical indication of the form DNA-dependent DNA-polymerases and its function, as an example of application of α-amanitine for determination of DNA-dependent RNA-polymerase II (B) and its differentiation from I (A) and III (C) forms of these enzyme [4, 5].

Determination of 3′→5′ DNA-dependent DNA-polymerase function by a direct method is know to be quite a complicated and difficult procedure [6, 7]. Otherwise, the combination of doxorubicin and streptonigrin can be used for selectivity inhibition of the function of one or another DNA-polymerase in the assay where both enzymes are in the mix.

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НЕПРЯМЕ ВИЗНАЧЕННЯ 5′→3′ І 3′→5′ ФУНКЦІЙ ДНК-ЗАЛЕЖНИХ ДНК-ПОЛІМЕРАЗ

Резюме

Доксорубіцин в концентрації 1 мкг/мл і стрептонігрин в концентрації 1000 мкг/мл кожний окремо можуть застосовуватися для визначення реплікативної (5′→3′) або репаративної (3′→5′) функцій ДНК-полімерази. Активність ферменту з реплікативною (5′→3′) функцією повністю пригнічується доксорубіцином, але не чутлива до дії стрептонігрину в концентраціях, зазначених вище. І, навпаки, активність ДНК-полімерази з репаративною (3′→5′) функцією не чутлива до впливу доксорубіцину, але інгібується стрептонігрином.

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