

Interaction of Netropsin with Double-Stranded Nucleic Acids Irradiated with Nonionizing Athermal Millimeter Electromagnetic Waves

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Abstract. The binding of antitumor drug netropsin with natural and synthetic double-stranded nucleic acids was investigated. Nucleic acids were irradiated by non-thermal millimeter coherent electromagnetic waves with resonant (64.5 and 50.3 GHz) and non-resonant (48.3 GHz) frequencies of water structures. Studies demonstrate that absorption curves of the complexes and the character of change during titration process are the same for irradiated and non-irradiated DNAs. The results show that binding constant (K) of netropsin with irradiated DNAs changes: almost by one order for B-form DNA (extracted from calf thymus) and is tripled in case of A-form DNA (poly(A)poly(U)). The changes of enthalpy (ΔH) and entropy (ΔS) for binding process were calculated by Van't Hoff analysis from the dependence of K on the temperature. As a result of irradiation with resonant frequencies, increase in absolute values of ΔH and ΔS takes place, while in case of non-resonant irradiation these values practically remain constant.

Keywords: Double-stranded nucleic acids, B- and A- conformations, netropsin, millimeter electromagnetic waves.

Introduction

A large class of compounds binding to DNA in non-intercalating way comprises of netropsin and distamycin derivatives [1,2]. They are more or less linear, flexible molecules bearing a positive charge on one (or two) end of the molecule having various functional substituents, such as H-linking donors (acceptors) in the middle part of the molecule. Netropsin shows AT-specificity upon binding with DNA in its narrow groove, forming an ordered complex, which properties are highly dependent on AT content (assuming that the interaction is carried out with a sequence of 3-5 AT bases pairs) [3-8].

Overviewing the literature data on binding the antitumor compounds netropsin with DNA, we can conclude that netropsins specifically interacts with AT-pairs of the DNA B- form in intercalating way, locating in a narrow groove. Positively charged NH_2 groups electrostatically interact with the DNA phosphates and peptide groups forming three hydrogen bonds with O_2 atom of thymine and with N_3 atom of adenine [4-7].

We have shown that at irradiation of DNA solutions by non-thermal coherent millimeter (MEM) waves, which are resonant for water structures, the physico-chemical characteristics and the thermal stability of the DNA solutions are changed, which are caused by dehydration of DNA and metal ions [9-11]. Therefore, in this work we investigated the interaction of non-intercalating anticancer drug netropsin with DNA, preliminary irradiated by resonant (50.3 GHz and 64.5 GHz) and non-resonant (48.3 GHz) frequencies with water structures oscillation.

Materials and methods

We used the DNA isolated from calf thymus, synthetic polyribonucleic acids poly (A) poly (U), which is always in A-conformation [12]. Studies were carried out in 0.1 M Trisbuffer, pH 7.5, at 20, 30 and 40°C. Under these conditions, calf thymus DNA is in B-conformation [12]. Concentrations of netropsin and nucleic acids were determined using the following values of molecular extinction coefficient in $M^{-1} cm^{-1}$ units. For calf thymus DNA $\epsilon_{260} (P) = 6550$, poly (A) poly (U) $\epsilon_{260} (P) = 7140$, netropsin $\epsilon_{296} (P) = 21500$. Netropsin-nucleic acids complexes were studied by spectrophotometric method. The absorption spectra were obtained using SPECORD UV VIS spectrophotometer. Irradiation of solutions by MEM waves was carried out in a special glass vessel, detail description of which is given in [9]. GI-142 and GI-141 were used as radiation generators. Interval of oscillation frequency for the GI-141 is 37.5-53.57 GHz (flux density – 0.6 mVt/cm²), and for the GI-142 is 53.3- 78.33 GHz (flux density- 50mcVt/cm²). Solutions of the nucleic acids were irradiated for 90 min by 50.3 and 64.5 GHz frequencies, which coincide with the resonance frequencies of oscillation of water molecular structures, as well as by 48.3 GHz non-resonant frequency. As it, in [10] was shown the maximum change in the physicochemical properties of DNA occurs at irradiation of DNA solution for 90 min.

Results and discussion

Absorption spectra of non-irradiated and irradiated for 90 minutes calf thymus DNA and synthetic polyribonucleotide poly (A) poly (U) with netropsin have been obtained. Experiments show that the absorption spectra of complexes and the nature of their change at titration are almost the same for the non-irradiated and irradiated nucleic acids. From the absorption spectra concentration of free and bound netropsin in solution was determined, and isotherms of binding were drawn according to the method described in [13].

The binding isotherms (Figs. 1 and 2) are described by Eq. (1), which more accurately describes the adsorption of low molecular weight compounds in the double-stranded nucleic acid:

$$\frac{r}{C_f} = K(1 - nr)^n [1 - (n - 1)r]^{1-n} \quad (1)$$

where $r = C_b/C_p, C_b$ and C_f concentration of bound and free netropsin solution, C_p - concentration of nucleic acids per base pair, parameters K and n describe the complex, K - binding constant, n - parameter characterizing stoichiometry of the polymer-ligand complex at saturation and equal to the number of bases pairs of the polymer occupied by one bound ligand molecule.

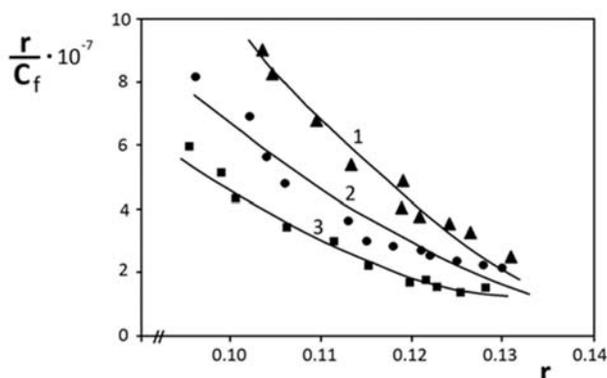


Fig. 1. The binding isotherms are drawn using spectrophotometric titration of netropsin with calf thymus DNA at temperatures of 20 (1), 30 (2) and 40 °C (3).

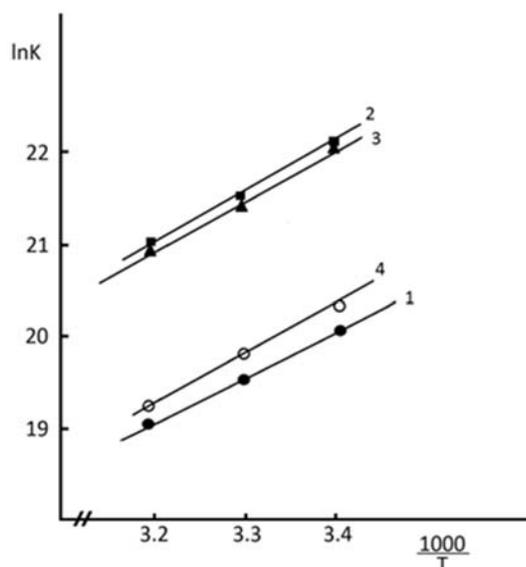


Fig. 2. $\ln K$ dependence at $1/T$, calculated using binding isotherms of netropsins with DNA non-irradiated and irradiated by MEM waves with of 64.5 (2) 50.3 (3) and 48.3 GHz (4) frequencies.

Figs. 1 and 2 show the binding isotherm of netropsin with calf thymus DNA and poly (A) poly (U) in Scatchard coordinates at temperatures of 20, 30, 40°C. The solid line is a theoretical curve drawn through the experimental points by the method of least squares, which satisfies the Eq. (1).

First, let us consider binding netropsin with non-irradiated DNA and RNA. The value of parameters K and n for complexes netropsin with calf thymus DNA (B-form) and synthetic double stranded polyribonucleotide poly (A) poly (U) (A-form) at different temperatures, calculated by formula (1) are shown in Table. 1.

As can be seen from Table 1, K is almost 4 times more when bound to the B-form than bound to the A-form: netropsin with more hydrated B-form forms more stable complex, than withless hydrated A- form. For netropsin-DNA complexes – $n \approx 6$, and for netropsin-RNA complexes – $n \approx 8$. Most likely, the difference of the values of n and K , obtained for DNA-netropsin and RNA– netropsin is due to the fact that under the same external conditions the DNA is in B-form, and the RNA is in A-form, which significantly differ in the helix geometry and hydration. In addition, the uracil in RNA is replaced by thymine, which greatly affects on stability of the double helix.

The values of parameters K and n are consistent with those of other authors [2,3] obtained for netropsin binding with DNA.

Table 1. Thermodynamic parameters of netropsin binding with non-irradiated double-stranded nucleic acids at several temperatures.

T, K	$K \cdot 10^{-8}, M^{-1}$	$-\Delta G$ kcal/mol	n
DNA - netropsin			
293	5.0 ± 0.2	11.7 ± 0.2	6.0 ± 0.1
303	3.0 ± 0.2	11.7 ± 0.2	5.9 ± 0.2
313	1.9 ± 0.2	11.9 ± 0.2	6.0 ± 0.2
poly(A)poly(U) – netropsin			
293	1.20 ± 0.02	10.9 ± 0.2	8.0 ± 0.1
303	0.72 ± 0.03	10.9 ± 0.2	8.1 ± 0.2
313	0.44 ± 0.02	11.0 ± 0.2	8.0 ± 0.2

For irradiated with resonant 64.5 and 50.3 GHz frequencies for water structures, the binding constant, which characterizes the strength of the complexes of irradiated nucleic acids with netropsin, almost is of one order of magnitude greater for the B-form, and 3 times greater for A-form (Table 2). When irradiation of solutions of calf thymus DNA and poly (A) poly (U) with non-resonance frequency (e.g., 48.3 GHz), the thermodynamic parameters characterizing the complexation of double-stranded nucleic acids with netropsin are constant are constant within accuracy of the experimentally calculated values (Tables 1 and 2).

Table 2. Thermodynamic parameters of netropsin binding with irradiated double-stranded nucleic acids at several temperatures.

The radiation frequency	T, K	$K \cdot 10^{-8}, M^{-1}$	$-\Delta G$ kcal/mol	n
DNA - netropsin				
50.3 GHz	293	36.1±0.3	12.8±0.3	5.9±0.2
	303	21.1±0.2	12.9±0.2	6.0±0.2
	313	12.8±0.3	13.1±0.3	6.0±0.2
64.5 GHz	293	38.4±0.2	12.9±0.3	6.0±0.2
	303	22.3±0.3	13.0±0.2	6.1±0.2
	313	13.0±0.2	13.1±0.3	6.1±0.2
48.3 GHz	293	6.9±0.2	11.9±0.2	6.0±0.3
	303	4.1±0.2	11.9±0.2	6.2±0.3
	313	2.4±0.2	12.0±0.2	6.1±0.2
poly(A)poy(U) – netropsin				
50.3 GHz	293	3.9±0.2	11.5±0.1	8.0±0.2
	303	2.3±0.1	11.6±0.2	8.1±0.3
	313	1.4±0.1	11.7±0.1	8.0±0.2
64.5 GHz	293	4.2±0.1	11.6±0.2	8.0±0.2
	303	2.5±0.1	11.6±0.2	8.0±0.2
	313	1.5±0.1	11.7±0.2	8.2±0.2
48.3 GHz	293	1.71±0.05	11.1±0.2	7.9±0.3
	303	1.02±0.05	11.1±0.3	8.2±0.3
	313	0.63±0.03	11.2±0.2	8.0±0.2

In [10,14], in explaining the experimental data, it was assumed that irradiation by the resonant for water structures frequencies hydration of Na^+ ions presented in the solution and bases pairs of double-stranded DNA decreases. Consequently, most of all, when irradiated nucleic acids with resonant 50.3 and 64.5 GHz frequencies in a hydrated B-form much severe dehydration, whereby, netropsin locating in a narrow groove, is able to bind more tightly with its positively charged NH_2 groups with negatively charged phosphate groups of nucleotides, which leads to an increase in the binding constant.

The value of change of the Gibbs free energy (ΔG), calculated according to the formula (2), for the investigated complexes are also given in Tables 1 and 2.

$$\Delta G = -RT \ln K \quad (2)$$

where R - gas constant, T - absolute temperature. The change of enthalpy (ΔH) and entropy (ΔS) at binding netropsin with the double-stranded nucleic acids has been determined from the analysis of Van't Hoff's K dependence on temperature.

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

where, taking into account (2) expression can be represented as follows

$$\ln K = -\frac{\Delta H}{R} \frac{1}{T} + \frac{\Delta S}{R} \quad (4)$$

According to the formula (4), the tangent of slope angle of $\ln K$ curve depending on $1/T$ gives the $\Delta H/R$ value, if the relationship is linear, and the ordinate of intersection with the axis of ordinates - $\Delta S/R$.

In each graphs, by the method of least squares through three experimental points, the straight line was drawn from which the values of ΔH and ΔS were determined and listed in Table. 3.

Fig. 2 shows the dependence of $\ln K$ versus $1/T$ for studies of non-irradiated and irradiated DNA complexes.

It should be noted that in Marks et al. work [2] for binding netropsin with DNA the similar values for ΔH and ΔS were found.

Table 3. The values of enthalpy (ΔH) and entropy (ΔS) of netropsin binding with irradiated and non-irradiated double-stranded nucleic acids.

Thermodynamic parameters	Non-irradiated	Irradiated, with frequencies (GHz)		
		50.3	64.5	48.3
DNA – netropsin				
$-\Delta H, \frac{\text{kcal}}{\text{mol}}$	9.1±0.2	9.5±0.2	9.6±0.2	9.2±0.2
$\Delta S, \frac{\text{cal}}{\text{mol} \cdot \text{K}}$	8.6±0.2	11.2±0.2	11.2±0.2	8.9±0.2
poly(A)poly(U)-netropsin				
$-\Delta H, \frac{\text{kcal}}{\text{mol}}$	9.1±0.2	9.3±0.2	9.3±0.2	9.1±0.2
$\Delta S, \frac{\text{cal}}{\text{mol} \cdot \text{K}}$	5.9±0.2	7.6±0.2	7.6±0.2	6.6±0.3

Table 3 shows that the irradiation with resonant frequencies increases ΔH and ΔS values, which is more pronounced for binding netropsin with calf thymus DNA (B-form). Irradiation of the natural and synthetic double-stranded nucleic acids by the non-resonant

frequency (48.3 GHz), values of binding enthalpy (ΔH) and entropy (ΔS) within the determination accuracy almost do not change.

Therefore, when netropsin binds with double-stranded nucleic acids irradiated with resonant frequencies the thermodynamic binding parameters are changed, where the value of the changes mainly depends on the conformational state of the double-stranded nucleic acids. The increase in the thermodynamic binding parameters (K , ΔH , ΔS) at complexing of anticancer drug netropsin *in vitro* with irradiated double-stranded nucleic acids indicates of prospects of development of the complex millimeter therapy with anticancer drug for clinical oncology in the treatment of cancer tumors.

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