PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Physical and Mathematical Sciences 2014, № 1, p. 51–56

Physics

INFLUENCE OF CATIONIC PORPHYRINS ON MELTING PARAMETERS OF CALF THYMUS DNA

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The influence of water soluble cationic 3N- and 4N-pyridyl porphyrins with different peripheral substituents on melting parameters of Calf Thymus DNA has been studied. It was shown, that the presence of porphyrin changes the shape and parameters of DNA melting curve. The decrease of ΔT in the presence of 3N-porphyrins is observed. Because of the intercalation binding mechanism occurs in GC-rich regions of DNA, which is the reason for the decrease melting interval. While even at the low relative concentration for 4N-porphyrins already the external binding mechanism "turns on" and as a result the change in the melting parameters of DNA upon complexation with these porphyrins is not observed.

***Keywords*:** Calf Thymus DNA, porphyrin, melting curves, melting parameters.

Introduction. It is known that during the biological functioning of DNA a partial opening of double helix (the so-called local melting) takes place. It is supposed that some anticancer compounds, such as porphyrins, interact directly with DNA of tumor cells. Therefore, the influence of anticancer drugs on DNA melting is still an actual problem. The interactions of porphyrin molecule and its many analogues have been studied since 1979 [1]. Having a pronounced anti-tumor, anti-viral and antibacterial properties, these compounds are in the focus of many scientists and research laboratories.

As the nucleic acids are a possible target objects for photodynamic influence studies on cells [2] and finally a huge clinical potential of porphyrins [3] led to many studies of the interaction of the cationic water-soluble porphyrins with a DNA poly-, oligo- and mononucleotides. Since then much progress has been achieved in studies of the complexation of porphyrins with native DNA.

The principal works in this field of research have been made for water-soluble cationic meso-tetra(*n*-N-methylpyridyl)porphyrins – TMPyP. For investigation of complexation mechanisms different methods have been used: the absorption and IR spectroscopy, circular dichroism spectroscopy, fluorescence, NMR and EPR

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methods, quantum chemical calculations, etc. Surly, the main proof of a binding mechanism studies is given by X-ray structure analysis of complexes of porphyrins with oligonucleotides [4].

The binding mechanism of porphyrin with DNA depends on various factors like the structure of DNA, porphyrin structure and the properties of the solution [5–7]. The main factor influencing the mechanism of porphyrin binding to DNA is the presence and type of peripheral radicals of porphyrins: their size, charge distribution and mobility, etc. A small change of components and structure of peripheral radicals may change the strength and mechanism of interaction. In [8] a binding mode can be programmed by varying the alkyl/aryl radicals.

The position of methyl group on pyridyl ring also influences on the interaction mechanism of porphyrins with DNA [9]. It has been shown, that if 4N-methylpyridyl porphyrin intercalate between the base pairs of double-helix of DNA, then its 2N-methyl-pyridyl analogue is unable to intercalate and binding occurs only by external mode, while the 3N-analog occupies an intermediate position.

No less important influences on the binding type is exerted by the relative concentration of porphyrin: v = [porphyrin]:[DNA]. It was shown that at relatively low values of v, TMPyP porphyrin intercalate into DNA, mainly in GC-rich regions [10], the increasing of relative concentration leads to a second binding mechanism – external orderly [11]. Even for porphyrins, which at low concentrations are associated with an external disordered mode in the groove of DNA, the increasing of relative concentration can lead to the formation of external ordered stacking on the DNA surface.

In our laboratory we continue intense research concerning the influence of peripheral group structure on the formation of a complex of porphyrins with DNA, that is carried out by means of absorption and CD spectroscopy [7, 9, 12, 13].

In the present study were investigated the effect of water soluble cationic 3N- and 4N-pyridyl porphyrins with different peripheral substituents (oxyethyl, buthyl, allyl, metallyl) on the melting of Calf Thymus DNA by thermal denaturation, also the earlier obtained results for the full effect of these porphyrins on the thermal stability of DNA are summarized.

Materials and Methods. Ultrapure Calf Thymus DNA (protein < 0.1%, RNA < 0.2%, M.w. > 30 *MDa*: GC=42%), kindly provided as a gift by prof. D.Yu. Lando from the Institute of Bioorganic Chemistry (Minsk, Belarus) was used in the experiments. Porphyrins were synthesized in the Department of Pharmacological Chemistry, Yerevan State Medical University by the method described in [14].

The melting experiments were performed in 0.1 BPSE buffer (ionic strength μ = 0.02 *M*). The pH of the solutions used throughout the experiments was kept constant at 7.4±0.1. A stock solution of DNA was prepared in 10^{-3} *M* NaCl solution at pH 7.4. An extinction coefficient $\varepsilon_{260} = 1.31 \cdot 10^4\ M^{-1}cm^{-1}$ was applied to determine the concentration of DNA in base pairs. The porphyrin solutions were prepared before each experiment and kept in the dark. Melting curves were recorded on Perkin Elmer Lambda 800 UV/VIS spectrophotometer with heating rate $1°C\ min^{-1}$.

Results and Discussion. To investigate the effects of porphyrins on DNA thermostability, DNA–porphyrin thermal melting experiments were performed. The melting temperature (T_m) of DNA is known to be sensitive to the double helix stability and varied depending on the strength of interactions owing to the binding

of compounds with DNA [15]. Therefore, T_m can be used as an indicator of binding properties of molecules to DNA and their binding strength.

To study the variation of thermostability upon interaction of investigated porphyrins with DNA, T_m was measured at v=0.01, 0.05 and 0.1. It appeared that for some porphyrins at relative porphyrin concentrations v=0.1 the melting process of DNA–porphyrin complexes finished above 98°*C*, owing to which the recording of entire melting process failed, and, hence, an accurate determination of melting curve parameters under these conditions proved impossible. The melting parameters of DNA in the presence of tested porphyrins for three different relative concentrations are summarized in Table.

The melting temperature (T^*_m) and melting interval (ΔT^{**}) at λ=260nm of DNA–porphyrin complexes

Porphyrins	v = 0.01		v = 0.05		v = 0.1	
	T_m, °*C*	ΔT, °*C*	T_m, °*C*	ΔT, °*C*	T_m, °*C*	ΔT, °*C*
TOEPyP4	71.7	10.5	74.4	14	–	–
TButPyP4	71.8	10	75.6	14.1	–	–
TAlPyP4	73.1	11.2	76.8	15.6	–	–
TMetAlPyP4	71.9	10.9	76.3	14.2	–	–
TOEPyP3	71	7.3	72.8	11.8	75.8	15
TButPyP3	71.8	10	74.3	13.3	75.3	13.9
TAlPyP3	71.3	7.45	74.6	14.9	75.1	14.9
TMetAlPyP3	71.4	7.7	–	–	75.2	12.7

Note: the melting parameters of pure DNA are: $T_m = 71.9°C$, $\Delta T = 10.4°C$;
* T_m is midpoint of melting curve; ** $\Delta T = \left(\partial\theta/\partial T\right)^{-1}_{\max}$.

In Fig. 1 are shown the melting curves of complexes of DNA with two different porphyrins, as a melting curve sample.

It follows from the analysis of data, that at low relevant concentration v=0.01 T_m of complexes practically did not change according T_m of pure DNA. For 4N-porphyrins ΔT did not changes also, meanwhile for 3N-porphyrins ΔT was observed to increase. Analyze of melting curves (not presented here) of this relevant concentration the 3N-porphyrins selectively destabilize GC-base pairs of DNA (the end of melting temperature shift to the range of low temperatures, than the beginning of the curve). It is known that the influence of selective binding of ligands with base pairs on T_m of DNA is weak [15], those ΔT of DNA is sensitive to selective binding. The ligands destabilizing GC-base pairs decrease the value of ΔT more, than the non-selectively binding ligands.

Thus, the fact of melting range decrease permit to conclude that the 3N-analogues of the metal-free porphyrins show selectively destabilization of GC-DNA region. Since the binding by intercalation mechanism generally occurs in GC-rich regions of DNA, the obtained results can be explained as follows: 3N-porphyrins, intercalated in GC-rich regions, reduce the thermal stability of these sites, bringing them closer to the thermal stability of AT-regions, which is the reason for the decrease the melting range. For such low concentrations of the investigated 3N-porphyrins an intercalation mode of binding is realized, that was mentioned in our previous works on interpretation of DNA–porphyrin complexes

CD spectra. As for 4N-porphyrins in case of v=0.01, the external mechanism of binding is probably already turned on, and destabilizing effect of porphyrins on GC-base pairs of DNA is compensated the stabilizing effect on AT-pair, whereby changes in the melting interval of DNA upon complexation with porphyrins are not observed.

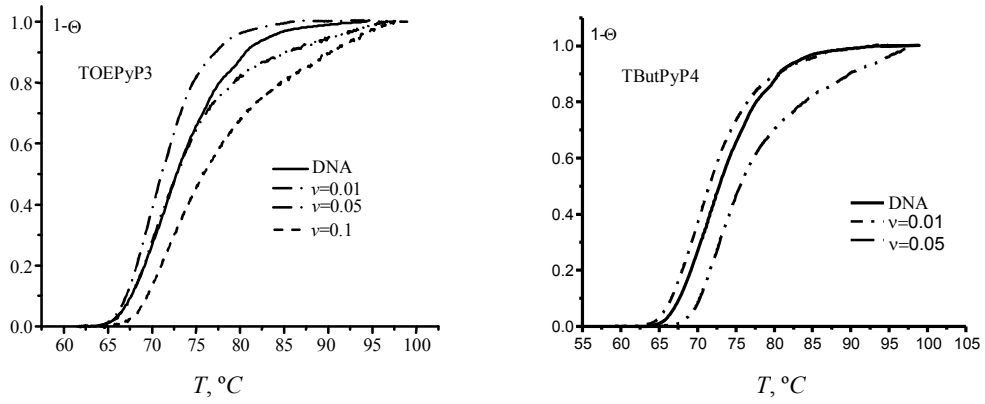

Fig. 1. Thermal denaturation of DNA alone in the presence of TOETPyP3 porphyrin and TButPyP4 porphyrin at different relevant concentrations.

The stabilizing effect of porphyrins on AT-pairs can be explained by the fact that at external binding the porphyrins form stack structures on DNA (in AT-rich regions) and an extra energy is needed for melting them, which leads to an increase in thermal stability of AT-base pairs. The observed increase in T_m and ΔT at relative concentrations of porphyrins in excess of 0.01 is most probably related to the additional energy required for destruction of internal and external stacking porphyrins.

Furthermore, as is seen from Table, at the same concentrations of porphyrins T_m of DNA complexes with 4N-porphyrins is higher than that of complexes with 3N-porphyrins. The values of the melting parameters of DNA in the presence of 4N-porphyrins at relative concentration v=0.1 are not shown in the Table, because at these concentrations these porphyrins so strongly stabilize the structure of DNA, that it proved impossible to register the end of complexes melting.

In Figs. 2 and 3 the melting curves of complexes DNA with 3N- and 4N-porphyrins are presented at different relative concentrations. As is seen in Fig. 2, a, the selective destabilization of GC-rich DNA regions for 3N-porphyrins is observed at low concentrations. This can be explained by the fact that at the given relative concentration these porphyrins intercalate in GC-regions, thereby causing local destabilization of the structure DNA at binding sites. For 4N-porphyrin, at the same concentrations no definitive conclusions about the places of selective binding may be done (Fig. 3, a).

The stabilization of AT- and GC-pairs is observed by increasing the concentration as the 3N-porphyrin (Fig. 2, b and c), so the 4N-porphyrin (Fig. 3, b), but stabilization of GC-pairs is more advantageous. The increase of ΔT indicate that the binding mechanism with DNA for these porphyrins preferably the intercalation (in GC-rich regions).

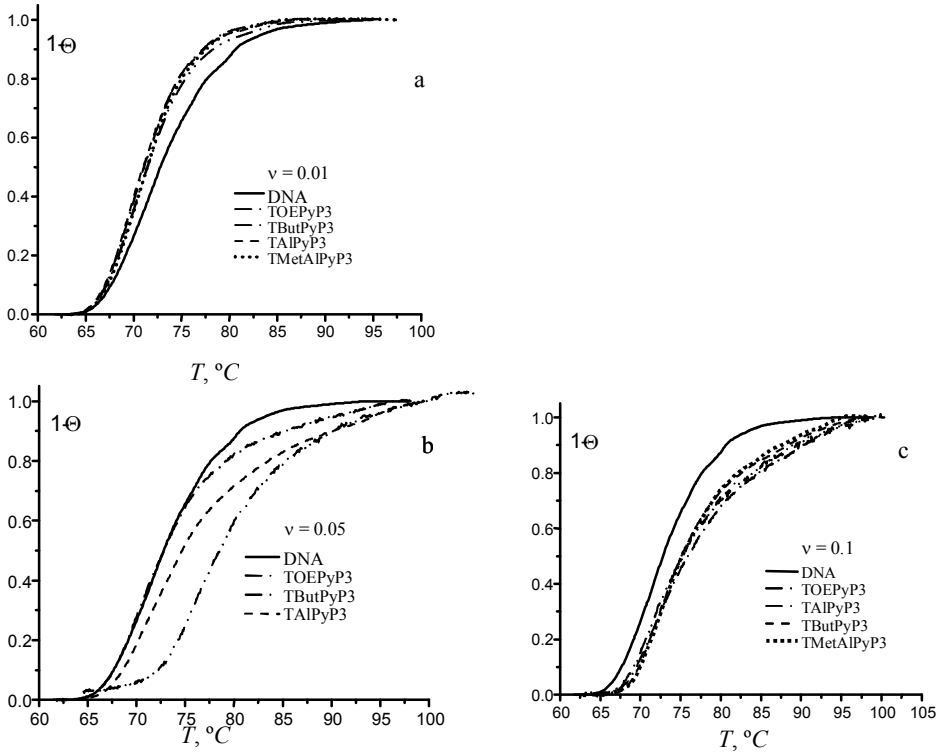

Fig. 2. Melting curves of DNA in the presence of 3N-porphyrins:
a) $\nu = 0.01$; b) 0.05; c) 0.1.

b

Apparently, at low concentrations of porphyrins, when one porphyrin molecule is related with about 100 base pairs, the intercalation leads to a local disruption of the structure of DNA at the binding sites, which breaks DNA based pairs stacking. While at higher concentrations the extended stacks of intercalated porphyrins are formed, leading to an increase in the thermal stability of the complex, due to the stacking interaction of neighboring porphyrins one with the other.

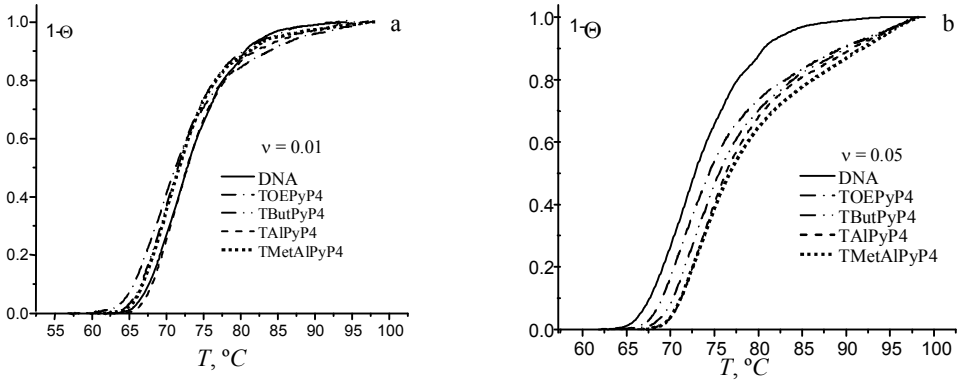

Fig. 3. Melting curves of DNA in the present of 4N-porphyrins: a) ν = 0.01; b) ν = 0.05.

Conclusion. By obtaining the thermal denaturation curves as well as summarizing the earlier results obtained by means of other methods the effect of cationic porphyrins with different peripheral substituents on the thermal stability of DNA has been evaluated. It is shown, dependance of the effect to on the relative concentration of porphyrins and the position of peripheral groups on pyridyl ring of porphyrin (3N or 4N). Also it should be noted, that depending of the type of peripheral substituents the effect on DNA melting parameters is individual for each porphyrin under investigation.

Received 20.12.2013

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