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Microcalorimetric study of DNA–Cu(II)TOEPyP(4) porphyrin complex

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Abstract The influence of new water soluble cationic metalloporphyrin Cu(II)TOEPyP(4) (meso-tetra-(4-*N*-oxyethylpyridyl)), analogue of Cu(II)TMPyP(4), on thermodynamic stability of DNA at various molar ratios of $r =$ porphyrin/DNA b.p. ($0 < r < 0.12$) has been studied. It has been shown that Cu(II)TOEPyP(4) is a strong stabilizing agent for calf thymus DNA increasing its melting temperature from 75.5 to 99.5 °C, in the range $0 < r < 0.06$. The melting enthalpy (ΔH_m) does not change in the range $0.002 < r < 0.06$ and it equals to 11.6 ± 0.8 cal/g. At $r > 0.07$, ΔH_m and T_m decrease, and at $r = 0.12$ they equal to 6.4 ± 0.6 cal/g and 92.5 °C, accordingly. We suggest that such centers of binding are the well documented 5′CG3′ sites and G-quadruplex at $r < 0.01$, and negatively charged phosphate groups at $r > 0.01$. On the basis of ΔH_m invariability with simultaneous increase of T_m in the range $0.002 < r < 0.06$, it is shown that the DNA-Cu(II)TOEPyP(4) complex melting is not of an enthalpic nature but of an entropic one. The two-phase helix–coil transition of DNA at $r < 0.01$ is considered as a result of porphyrin redistribution in the melting process.

Keywords Microcalorimeter · Metalloporphyrin · DNA–porphyrin complex melting

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Introduction

During the past decade, a significant progress has been achieved in synthesis of new small molecules that can bind to DNA at selected sites, and can interfere with transcription, DNA replication, and reparation processes. They have numerous applications in gene analysis, diagnostics, therapeutics, study of DNA conformations, and nanotechnology [1–5]. In this respect, a reversible binding of water soluble cationic anti-carcinogenic porphyrin Cu(II)TOEpyP(4) (meso-tetra-(4-*N*-oxyethylpyridyl), which is analogue of Cu(II)TMPyP(4), to DNA [4–8] is of a significant interest due to the fact that these planar small quadrangular ligands penetrate into tumor cells nuclei [7], bind to DNA quadruplex and inhibit tumor growth in vivo [2, 6].

These structures of porphyrin–DNA complexes have been extensively studied with various physical techniques [8–14]. It was determined that planar (four-coordinated) metalloporphyrin Cu(II)TMPyP fulfills several modes of binding with GC and AT pairs of DNA in water solutions, namely, (a) a "strong but seldom" intercalation only into 5′CG3′, but neither 5′GC3′ nor any other sequences [12], and a surface intercalation into the G-quadruplex [5, 7]; and (b) a "weak but frequent" outside groove binding at AT sites. This binding has several versions: (1) outside random binding—irregular groove binding, when the sides of the porphyrin ring fit into the minor groove of DNA or locate in the major groove by electrostatic interaction between the negatively charged phosphate group of DNA and the positively charged pyridyl rings of porphyrin; (2) specific binding due to absorption at the bottom of the major groove, when Cu(II)TMPyP(4) binds with at least four consecutively located AT pairs [11–13]; (3) regular binding—self-stacking mode at moderate and high values of *r*. In this case, porphyrin molecule planes are

perpendicular (or slightly deflected) to the DNA helix axis, and they are located over each other forming stacks with length of some tens of molecules [11–13]. This binding mode at high level of saturation of binding centers induces aggregation of DNA [14]. In spite of the significant success reached in understanding of binding modes of TMPyP(4) and TOPyP(4) and their metalloporphyrins with DNA [8, 11–14], no detailed thermodynamic analysis of melting process of these complexes has been carried out yet. In this study, we tried to obtain some new information about the melting of DNA in the wide range of molar ratio of porphyrin to DNA, with the help of differential scanning microcalorimeter (DSC) [15] which unlike the capillary DSC gives us a possibility to investigate dilute solutions, as well as suspensions and gels of biomacromolecules. The aim of this study is deeper understanding of physical mechanism of influence of Cu(II)TOEPyP(4) on DNA in solution. In particular, it is interesting to find out if it is a stabilizing or destabilizing agent for DNA, whether it mainly binds to AT or GC pairs, and what is its influence on melting energy. In our experiments, we use calf thymus DNA because the fact that calf thymus DNA melting mechanism has been studied in details in a wide range of pH, ionic forces of solutions, and in presence of various metal ions and porphyrins [16, 17]. Besides this, calf thymus DNA contains a GC-rich satellite fraction, which gives a clear peak on melting curve at heating. This fact may be used to see ligand influence on the certain sites with various GC content of the same DNA, and on the basis of the obtained data, we can judge about different influences of ligands on AT and GC pairs of DNA [16, 17].

Materials and methods

Ultra-pure DNA from calf thymus was used in our study (protein $< 0.5\%$, RNA < 0.2, $M_w > 20$ MDa, hyper-chromium effect $\sim 39\%$). All experiments were carried out in 20 mM phosphate buffer, pH 7.02. Porphyrin concentrations in solutions ranged from 1.8×10^{-4} to 1.3×10^{-6} M, DNA concentration was from 1.3×10^{-3} to 1.4×10^{-3} M. The water soluble cationic Cu(II)meso-tetra-(4-*N*-oxyethylpyridyl) ($M_w = 1044$ D) was synthesized and purified by Dr. R. Ghasaryan (Yerevan State Medical University, Armenia[8] and kindly supplied for our experiments (Fig. 1).

The measurements were performed using a DSC that gives a possibility to measure suspensions of cells, as well as dilute solutions and gels of biopolymers [15, 16]. The sensitivity of DSC is 0.1 μW, the volume of the measuring vessel is 0.22 cm^3, the heating rate is 0.6 °C/min, and the temperature interval of measurements is from 2 to 140 °C.

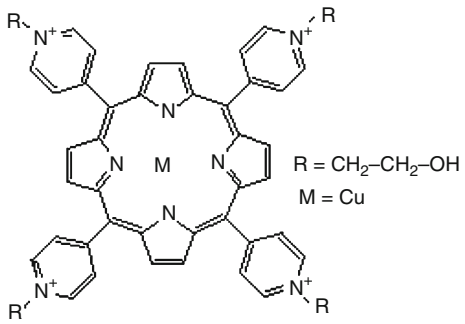

Fig. 1 Schematic structure of Cu(II)TOEPyP(4)

Results

Figure 2a,b presents the heat absorption curves of DNA solutions in the presence of different concentrations of Cu(II)TOEPyP(4). As it is seen, the DNA melting curve without porphyrin has a complex profile. According to [16], the main fraction of calf thymus DNA containing more AT pairs than satellite fractions melts at 78.5 °C, and melting of the satellite fractions is presented on the curve as a sharp peak at 85.9 °C and a shoulder at 90.0 °C (Fig. 2a).

A significant change of the melting curve profile and a shift of the curve to higher temperatures are observed already at $r = 0.002$, where r is the molar ratio of Cu(II)TOEPyP(4) to DNA b.p. The melting curve has a two-stage character in the range of r from 0.002 to 0.008. At $r = 0.007$, the first dominant transition ($T_m = 83.1$ °C) corresponds to the main fraction, the second one ($T_m = 94.5$ °C) corresponds to the satellite fraction that is formed due to the unification of a clear peak at 85.9 °C, and a shoulder at 90.0 °C. The shift of the satellite fraction to higher temperatures is more significant than the main fraction in this range of r, and this difference ($T_m^{sat} - T_m^{main}$) equals 6.5° at $r = 0$, and 11.5° at $r = 0.008$ (Fig. 3). The increase of porphyrin concentration (in the range of r from 0.008 to 0.06) causes further widening, smoothing and shifting of the curve to higher temperatures (Fig. 2b). The calculations of melting enthalpy from areas under the curves show that melting enthalpy of DNA in the range of r from 0 to 0.06 does not change and equals 48.50 ± 3.5 cal/g. With further increase of r, the enthalpy drops and equals 26.5 ± 2.5 cal/g at $r = 0.12$. These results and value of ΔS_m calculated from the dependence $\Delta S_m = \Delta H_m / T_m$, as well as T_m and ΔT_m at different r are presented in Figs. 4 and 5. As it is seen, $T_m(r)$ dependence has two-step character at $0 < r < 0.07$. On the first step ($0.002 < r < 0.01$), T_m increases by ~ 6.4 °C, and on the second one ($0.035 < r < 0.07$), T_m increases by ~ 14 °C (Fig. 4). In these cases, T_m is the temperature at which melting enthalpy is 1/2 value of the total melting enthalpy

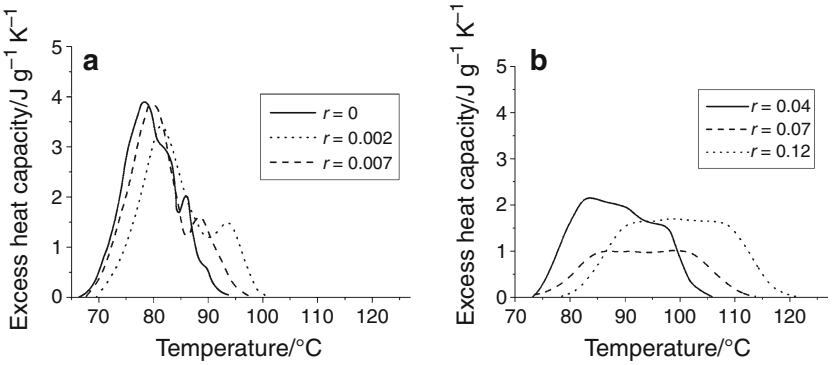

Fig. 2 Heat absorption curves of DNA-Cu(II)TOEPyP(4) complex at various values of r calculated per gram of DNA, in 20 mM Na-phosphate buffer, pH 7.02

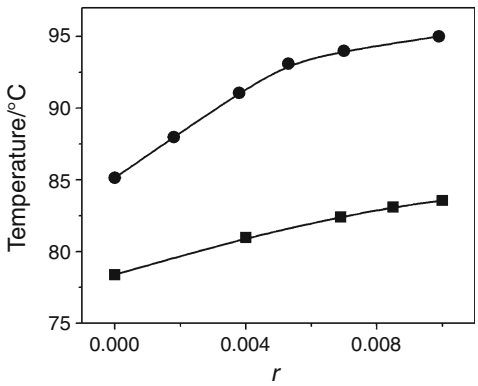

Fig. 3 The dependence of melting temperature (T_m) of main and satellite fractions of calf thymus DNA on the molar ratio of porphyrin to DNA b.p. (r) in case of the two-stage transition (20 mM Na-phosphate buffer, pH 7.02)

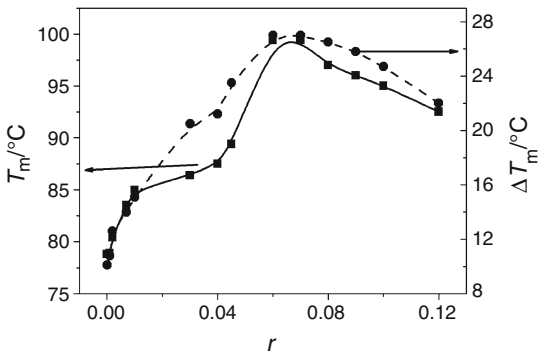

Fig. 5 The dependence of melting temperature and melting interval width of calf thymus DNA on the molar ratio of porphyrin to DNA b.p. (r). In this case, the melting temperature corresponds to the temperature at which the melting enthalpy equals 1/2 of the total enthalpy value. The experiments were carried out in 20 mM Na-phosphate buffer, pH 7.02

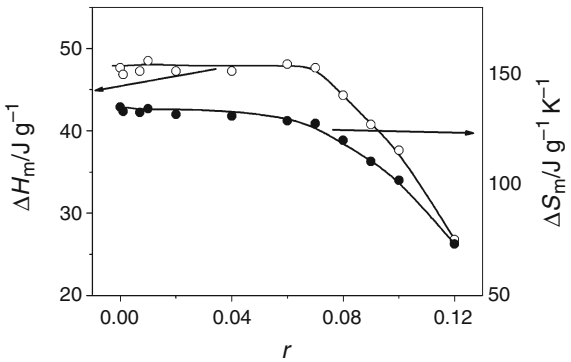

Fig. 4 The dependence of melting enthalpy (*open circle*) and entropy (*filled circle*) on r. The experiments were carried out in 20 mM Na-phosphate buffer, pH 7.02

(ΔH_m). Figures 4 and 5 also show that ΔH_m, ΔS_m, T_m, and ΔT_m of the complex decrease simultaneously in the range $0.07 < r < 0.12$.

Discussion

The data presented in Figs. 2 and 5 show that Cu(II)TOEPyP(4) is a strong stabilizing agent for calf thymus DNA, it causes a significant increase of DNA thermostability, broadening of the melting interval, and smoothing of the melting curve in the range $0.002 < r < 0.07$.

According to theoretical and experimental data [16, 17] on DNA helix–coil transition in the presence of small reversibly binding molecules, within the neutral pH range and at DNA/ligands ratio $r \ll 1$, DNA melting process has some peculiarities: (a) melting of DNA has a two-stage character due to ligand redistribution from melted regions to helical ones at $K2/K1 > 1$, where $K2$ and $K1$ are constants of binding with helical and melted regions; the two-stage character of melting is expressed the stronger the higher is $K2$ in comparison with $K1$; (b) in case of DNA stabilizing ligands, the increase of melting interval width is directly connected with stronger increase of GC pairs thermostability compared to AT pairs.

It is known that the coordinating number of Cu(II) is 4 and, due to this, Cu(II) in composition of TMPyP(4) and TOEPyP(4) forms coordinating bonds to four nitrogen atoms of porphyrin nucleus, and it is located in the porphyrin ring. This flat molecule intercalates between C and G pairs of DNA with binding constant (K) around 10^6 M^{-1}

in 5′CG3′ sites [12, 18], and around 1.6×10^7 M^{-1} in G-quadruplex [19]. It also binds to duplex via outside non-intercalative mode with $K < 10^6$ M^{-1} and melted sites with $K < 10^5$ M^{-1} [20]. Consequently, at extremely low values of r, AT-rich sites without porphyrin melt firstly, and then 5′CG3′ sites containing porphyrin and flanking AT-rich segments (e.g., ATATACGCGTATAT) melt [12]. As a result, porphyrin molecules released from 5′CG3′ sites will bind to G-quadruplex and then to 5′CG3′ sites with flanking GC sequences, and this is the reason for the two-stage process of DNA melting.

High values of the binding constant of Cu(II)TOEPyP(4) with rare GC-rich sites in comparison with the constant of outside groove binding must be determined not only by the two-phase helix–coil transition of DNA, but also by the stepped increase of T_m, depending on r, at $r << 0.07$ (Fig. 5). Actually, at extremely low values of r, increase of T_m by ~ 6.5 °C is possible only due to formation of strong porphyrin complex with 5′CG3′ sites and G-quadruplexes. In the range of r from 0.01 to 0.035, when these rare binding sites are occupied, T_m increases only by ~ 1.5 °C and its future increase by ~ 14 °C takes place only due to the other binding mode, in particular, outside random binding ($0.03 < r < 0.07$).

As Cu(II)TOEPyP(4) and its analogue Cu(II)TMPyP(4) are able to form a strong complex with GC b.p. via intercalation in 5′CG3′ sites, and more strong complexes with G-quadruplex TTAGGG, CCTAGG, and 5′-d(CGTACG) etc. [12], we suppose that T_m increase of satellite fraction in comparison with the main fraction in the range of r from 0.002 to 0.008 (Figs. 2, 3) is connected to the Cu(II)TOEPyP(4) complex formation at the sites of the abovementioned rare and unique sequences mainly located in GC-rich satellite fractions of calf thymus DNA rather than in the main fraction. The decrease of ΔH_m, ΔS_m, T_m, and ΔT_m at high degrees of binding centers filled with Cu(II) porphyrin ($r > 0.07$) takes place when Cu(II) porphyrins form stacks on the external surface of DNA duplex.

We attribute the decrease of melting parameters to strong structural changes initiated by the aggregation process, and this supposition coincides with results of the work, in which considerable structural changes of calf thymus DNA-Cu(II)TMPyP(4) complex at aggregation were observed using resonance Raman spectroscopy [14]. The invariability of ΔH_m with simultaneous increase of T_m in the range $0.002 < r < 0.07$ says that the complex melting in this range is not of an enthalpic but of an entropic nature. This conclusion is directly resulted from the dependence $T_m = \Delta H_m / \Delta S_m$, according to which T_m increase at constant ΔH_m is possible only due to decrease of ΔS_m. As Cu(II)TOEPyP(4) mainly binds to DNA duplex, the decrease of melting entropy ($\Delta S_m = S_{coil} - S_{helix}$) may happen at the expense of entropy of the helical state (S_{helix}) increase (S_{coil} is entropy of the coil state). It is supposed that S_{helix} increase is connected with forcing out of H_2O molecules from DNA duplex by Cu(II)TOEPyP(4). It is known that AT pairs contain more water than GC pairs. Besides this, water molecules in the AT-rich minor groove form a continuous spine, which results in stabilization of AT pairs [21]. Therefore, removing even a small portion of water molecules from outside groove where Cu(II) is bound to DNA may lead to disordering of the hydrate structure and this, in turn, may cause increase of entropy of AT pairs in helix state giving the main contribution to entropy helical state of the macromolecule. This conclusion coincides with data according to which Cu(II)TOPyP(4) molecule fits snugly into AT sites of minor groove with two inner *N*-methyl-pyridine groups oriented almost parallel to the groove floor, and Cu(II)TOPyP(4) excites the first-shell and the second-shell water molecules and this has significant effects on local and global DNA structure [10].

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