Journal of Inorganic Biochemistry 161 (2016) 83–90

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Surface complex of ZnTMPyP4 metalloporphyrin with double-stranded Poly(A)-Poly(U)

G. Tolstykh, V. Sizov, A. Kudrev *

Saint Petersburg State University, 7/9 Universitetskaya nab., St. Petersburg 199034, Russia

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 14 April 2016
Accepted 4 May 2016
Available online 6 May 2016

Keywords:
Zn(X)TMPyP4
Poly(A)-Poly(U)
Cooperativity

ABSTRACT

This communication presents synthesis and spectral characterization of metalloporphyrin [Zn(X)TMPyP4] (TMPyP4 is 5,10,15,20-tetrakis (*N*-methylpyridinium-4-yl)porphyrin), and studies its binding onto anionic surface sites of synthetic double stranded polynucleotide Poly(A)-Poly(U). [Zn(X)TMPyP4] binding with Poly(A)-Poly(U) was monitored by UV–Vis absorbance spectroscopy, two fluorescence spectroscopies and 1H NMR in a working aqueous medium of 0.15 M ionic strength, pH 7.0 and at 25 °C. The evidence provided by spectroscopic measurements and multivariate data analysis suggests the use of this metalloporphyrin as a probe for investigation of the polynucleotide surface. In contrast to TMPyP4 intercalation, an outside adsorption of [Zn(X)TMPyP4] induces an attenuation of luminescence intensity and has little influence on the shape of luminescence band. Special attention was paid to the quantitative description of the interaction between neighboring ligands on the Poly(A)-Poly(U) surface. The intrinsic binding constant to an isolated binding site lgK_{in} 5.8 ± 0.1, the cooperativity parameter ω 1.8 ± 0.2, and number of monomers occupied by a ligand n = 2 (25 °C; pH 7.0) were calculated based upon the recently proposed non-linear least-squares fitting procedure. The discovered cooperativity of binding of [Zn(X)TMPyP4] metalloporphyrin to Poly(A)-Poly(U) is significantly lower as compared to free porphyrin TMPyP4, reflecting minimal mutual influence between the nearest neighboring ligands bound with functional PO_4^- groups of the polynucleotide surface.

1. Introduction

A number of cationic metalloporphyrins MeTMPyP4 (Me = Ni(II), Pt(II), Pd(II), Cd(II), Pb(II), Cu(II), Co(II), Mn(II), Zn(II), Au(III), Bi(III), VO^{2+}, H_2TMPyP4 is 5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21H,23H-porphine that can nonspecifically interact to double-stranded (ds) DNA and RNA or bind selectively to specific DNA and RNA sequences have recently received considerable attention because of their potential applications in the development of anti-tumor drugs [1–11]. Furthermore, the medical relevance of cationic metalloporphyrins was stressed by the discovery that certain derivatives can inhibit telomerase activity [12,13]. In accordance with current views, this activity can be assigned to stabilization of the G-quadruplex DNA structures. It is believed that the insertion of the central ion in the porphyrin can help improve the selectivity of such reactions with biopolymers. This hypothesis was confirmed somewhat by the information that [MeTMPyP4] compounds were successfully used to target specific DNA motifs [14–16]. These findings are consistent with tentative conclusions derived from the footprinting studies demonstrating that groove-binding metalloporphyrins species protect AT-rich areas of DNA, e.g., when Me = Mn, Fe, Co and Zn [17]. On the other hand, Ni(II) and Cu(II) metalloporphyrins protect G-C rich domains [17,18].

Studies of the influence of the presence of a metal ion in the center of TMPyP4 core show that planar metalloporphyrins have a particularly high binding affinity for various DNA structures. This includes Ni(II), Pt(II), Pd(II), and Cu(II) porphyrins forming square planar complexes. The planar structures of cationic MeTMPyP4 allow interactions via π-stacking, as well as allow formation of supramolecular complexes in which porphyrins are electrostatically attached to phosphate groups. The molecular conformation of the porphyrin core varies slightly within this series of square planar complexes, so that the ability of a given porphyrin to intercalate may be correlated with the coordination ability of the central ion as well as with the structural lability of the bound macromolecule. However, geometrical distortion can arise owing to the orientation of peripheral substituents on the porphyrin ring or to the size of the metal center and its axial ligand. The planarity of the porphine ring and the effective width of the individual molecules increase when the central ion possesses a fifth and a sixth coordinated ligand [19]. It has been suggested that the octahedral six-coordinated Co(III) and

Abbreviations: TMPyP4, 5,10,15,20-tetrakis (*N*-methylpyridinium-4-yl)porphyrin; Poly(A)-Poly(U), Polyadenylnic-polyurridylic acid; mPAU, Monomeric unit of Poly(A)-Poly(U); K_{in}, Intrinsic binding constant; ds, Double-stranded; AMP, Adenosine monophosphate; UMP, Uridine monophosphate; ESI-MS, Electrospray ionization - mass spectrometry; DFT, Density functional theory; NMR, Nuclear magnetic resonance; PCA, Principal Component Analysis; CP, Conditional probabilities; MI, Mutual influence; SVD, Singular value decomposition.

* Corresponding author.
E-mail address: kudrevandrei@mail.ru (A. Kudrev).

http://dx.doi.org/10.1016/j.jinorgbio.2016.05.004
0162-0134/

Mn(III) derivatives of TMPyP4 bind to G4-DNA with lower affinity with respect to the planar porphyrins of the TMPyP4 series [20,21]. This conclusion can be supported by the suggestion that Mn(III) and Co(III) porphyrins carry two water molecules as axial ligands that are present on the metallocenter in aqueous solution that prevent intercalation between nucleic base pairs of DNA and reduces the DNA-porphyrin complex stability. From this point of view it was surprising that the complex [Zn(X)TMPyP4] containing H_2O as axial ligand was the most effective in stabilizing the G-quadruplex among the porphyrins tested [6,16,22].

It is a common and reasonable assumption that cationic porphyrin $[Zn(X)TMPyP4]^{4+}$ (in the following text the symbol of charge is omitted) and its analogs have a square pyramidal geometry (see Fig. 1), and one axial water molecule is coordinated to Zn(II) in aqueous solution [6,23–26]. The metal ions possess an out-of-plane position, therefore, causing a dome distortion because it coordinates axial ligand from only one side of the porphyrin plane. It is also worth noting that a square-planar structure, similar to that observed for natural porphyrins [27], was also considered in the literature for Zn(II) porphyrins [7,16,28]. Their dsDNA or ssRNA binding modes could be partial intercalation, groove binding, and outside or external binding of the porphyrin [29–31]. It is likely that the five-coordination property of Zn(II) should prohibits the intercalation of metalloprophyrin between dsDNA bases pairs. For ssRNA the binding mode has been hypothesized to be partial intercalation and phosphate binding, which lead to long-range conformational change [32]. The binding modes, as well as stability of adducts, depend on different factors such as the sequence and structure of DNA, the peripheral substituents on the porphyrin ring, and the structure of the solution [33–37]. In addition to the substituents, the local environment has a profound influence on the stability constants of supramolecular complexes. Yet the [Zn(X)TMPyP4] cation has been used as reference for estimating binding mode for several porphyrins from the results of spectroscopic experiments. The problem is that in practice the criteria intended for the conceivable binding mode(s) selection are usually insufficient.

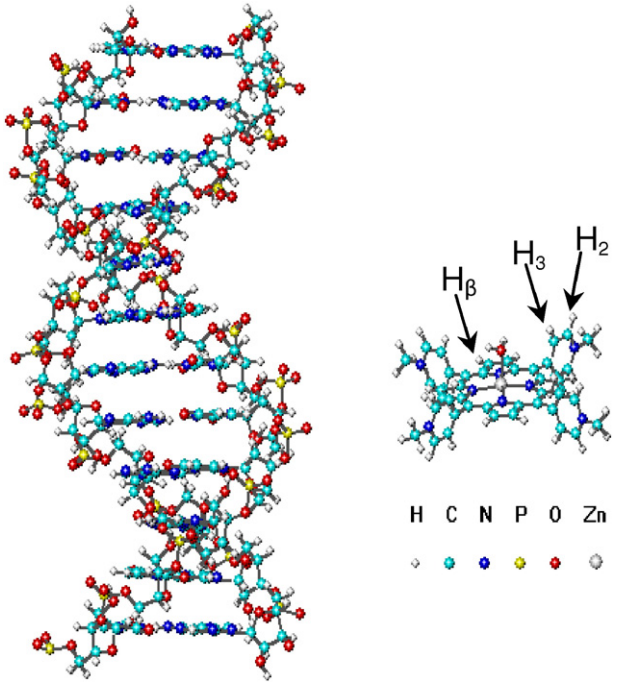

Fig. 1. Chemical structure of the Poly(A)-Poly(U) fragment and the structure of the $[Zn(H_2O)TMPyP4]^{4+}$ porphyrin complexes simulated by DFT calculations.

The interaction between [Zn(X)TMPyP4] and regular double-stranded polynucleotides was studied less extensively (see Table S1), and the binding mode has not yet been elucidated in detail [38,39]. A large planar π-system of [Zn(X)TMPyP4] can provide strong π-π stacking; on the other hand, cationic functional groups afford electrostatic interactions with phosphate groups on the surface of DNA. Among all metalloporphyrins, Zn(II)-derivatives show specific behavior since they can coordinate to anionic phosphate groups of the DNA rope thus causing additional stabilization of ds structure. However, coordination of metalloporphyrin to phosphate group raises the question of binding selectivity.

To our knowledge, the processes of binding [Zn(X)TMPyP4] with polyadenylnic-polyuridylic acid Poly(A)-Poly(U) were not studied until now. Previously reported data for systems containing [Zn(X)TMPyP4] or Poly(A)-Poly(U) are collected in Table S1. The possibility of interaction between metal complexes of TMPyP4 with Poly(A)-Poly(U) and conceivable binding mode(s) are still unrevealed. We are interested in performing a detailed study of the interaction between regular Poly(A)-Poly(U) and [Zn(X)TMPyP4] because in our experimental tests fluorescence quenching of [Zn(X)TMPyP4] has been detected in aqueous solution upon addition of the polynucleotide. This behavior contrasted with the reaction of Poly(A)-Poly(U) and free TMPyP4 which has been studied previously under identical conditions [37]. The hypothetical reason for such behavior is the difference in binding modes of these porphyrin derivatives governed by the five-coordination property of Zn(II).

The structural features of high polymeric synthetic double-stranded polynucleotide polyadenylnic-polyurridylic acid referred to as Poly(A)-Poly(U), which are caused by the relatively planar and equivalent stacking of aromatic bases along the helix sugar phosphate backbone (see Fig. 1), make it particularly interesting as a simplified model of natural biopolymers [40]. Moreover, high polymeric ribonucleotides were subject of investigation in connection with their biological activity *in vivo*; for instance, Poly(A)-Poly(U) is known as an inductor of *in vivo* interferon synthesis [41–43]. A synthetic Poly(A)-Poly(U) was shown to manifest both antitumor and immunomodulatory activities [44]. It seems probable that the activity of Poly(A)-Poly(U) is connected with reversible transformations in solution [45,46]. The possibility of regulating the activity by addition of reversibly binding complexing agents is very attractive. However, implementation of this approach is complicated by the lack of comprehensive thermodynamic models determining the state of the polynucleotide as function of other components' concentrations [47,48]. Therefore, investigation of the mechanism of complex formation between Poly(A)-Poly(U) and potential ligands is considered to be valuable for discussion of its biological activity *in vivo* and for drug design.

2. Materials and methods

2.1. Reagents

The following compounds were commercially available: potassium chloride (SIGMA), potassium hydroxide (CHEMAPOL), potassium dihydrogenphosphate (SIGMA), TMPyP4 tetratosylate (SIGMA), Poly(A)-Poly(U) acid (SIGMA), and $ZnCl_2$ anhydrous (REACHIM). All these compounds were used without further purification. Solutions of polynucleotide were prepared from a known amount of the solid reagent. Both Poly(A)-Poly(U) and metalloporphyrin solutions were prepared in phosphate buffer with pH 7.0 containing 0.15 M KCl. Concentration of the synthetic polynucleotide solutions refers to the concentration of the base pair of monophosphate nucleotides AMP and UMP, which is the monomeric unit in Poly(A)-Poly(U) chain. The total concentration of monomeric units of Poly(A)-Poly(U) available for binding of a ligand is C_{tMon}.