Interactions of Electronically Excited Copper(II)−Porphyrin with DNA: Resonance Raman Evidence for the Exciplex Formation with Adenine and Cytosine Residues

Peter Mojzeš,*,† Sergei G. Kruglik,‡ Vladimír Baumruk,† and Pierre-Yves Turpin§

Institute of Physics, Charles University, Ke Karlovu 5, CZ-12116 Prague 2, Czech Republic, B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, 70 F. Skaryna Avenue, Minsk 220072, Belarus, and L.P.B.C., Université Pierre et Marie Curie, mailbox 138, 4 Place Jussieu, F-75252 Paris 5, France

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Molecular complexes between the water-soluble cationic metalloporphyrin copper(II) 5,10,15,20-tetrakis[4-(*N*-methylpyridyl)]porphyrin (CuP) and a series of DNA-model single-stranded homopolynucleotides have been studied by resonance Raman spectroscopy as concerns their ability to form exciplexes under high-power nanosecond laser irradiation. Highly efficient exciplex formation is found for adenine-containing poly(dA) and cytosine-containing oligo(dC)$_9$. In contrast to thymine, uracil, and cytosine, adenine has no exocyclic C═O group which was presumed to participate in the CuP*C═O exciplex formation, and consequently one of its basic endocyclic nitrogens (N_1, N_3, or N_7) is proposed here to interact as an axial ligand with electronically excited CuP to form CuP*N exciplexes. The importance of an adequate geometry for CuP fixation to the polynucleotide for exciplex formation is also confirmed, since the exciplex is barely observed with poly(rA) and poly(rC), and even less in guanine-containing poly(dG) and poly(rG) despite the presence of C_6═O, N_3, and N_7 potential ligands.

Introduction

Water-soluble cationic (metallo)porphyrins complexed with nucleic acids (DNA, RNA) are widely investigated owing to their possible use in photodynamic therapy of cancer. To elucidate their binding modes to nucleic acids, investigation of porphyrins complexed with monomer nucleoside/nucleotide building units,[1,2] single-stranded[3−5] and double-stranded synthetic oligo/polynucleotides, and natural DNAs and RNAs (for a review, see refs 6−8) is of major importance.

Among various metalloporphyrins studied in complexes with nucleic acids, the copper(II) derivative of 5,10,15,20-tetrakis-[4-(*N*-methylpyridyl)]porphyrin (CuP) attracted particular interest owing to its ability to form in its excited electronic state a transient axially coordinated complex, the so-called exciplex, with a convenient ligand in its close proximity.[9−15] CuP exciplex formation can be conveniently monitored by resonance Raman spectroscopy (RRS), because, under high-power pulsed excitation, specific exciplex Raman bands appear at ∼1346 (ν_4*) and ∼1550 (ν_2*) cm^{-1} in pairs with their ground-state counterparts located at ∼1366 (ν_4) and ∼1570 (ν_2) cm^{-1}, respectively.[9] By analogy with (d,d)-excited copper(II) porphyrins (CuOEP, CuTPP) that were shown to form exciplexes with various oxygen-containing solvent molecules,[16,17] exocyclic keto substituents (C_2═O and/or C_4═O) of thymine (or uracil in RNA) have been proposed as the best candidates for axial ligation.[12,13] This is plausible since, in addition to double-stranded poly(dA-dT)$_2$, considerable yield of the CuP*C═O exciplex was found in CuP complexes with single-stranded poly(dT) and poly(rU).[12]

The presence of a convenient ligand is a necessary but not sufficient requirement for exciplex formation: the proper position of the porphyrin Cu center with respect to the potential ligand, conditioned by the porphyrin binding mode and by the overall secondary structure of the polymer, is also required for effective axial coordination. External groove-binding of CuP to runs of (at least four) alternating AT base pairs seems to provide the most favorable conditions for obtaining the exciplex,[11] while intercalation between base pairs disfavors or even fully hinders exciplex formation. For example, in contrast to strong exciplex RRS signals from complexes of outside-bound CuP with poly(dA-dT)$_2$, only weak exciplex signals were detected with poly(dA)·poly(dT).[11,12] Intercalation between GC base pairs (e.g. in poly(dG-dC)$_2$) completely prohibits exciplex formation, while weak but noticeable exciplex markers were found for CuP (hemi)intercalated into poly(dA-dC)·poly(dG-dT).[12,14] Recently it was shown[15] that exciplex formation is not restricted to regular polynucleotide structures, since a high yield of exciplex was observed in CuP molecular complexes with thymine mononucleotides (e.g. 5′-dTMP) in a large mononucleotide excess.

Besides the CuP*C═O species, a CuP*H_2O exciplex having a water molecule as its fifth axial ligand has been found in aqueous solutions containing free CuP molecules[13,14,18−20] Both exciplex species exhibit identical shifts of their RRS marker bands, but they can be distinguished by their relaxation kinetics in picosecond time-resolved RR (psTR^3) experiments,[14] since their lifetimes differ by about 3 orders of magnitude (∼1−3 ns and <10 ps, respectively).[14]

In the present study the question is addressed whether other bases, besides thymine and uracil, can give rise to the long-lived CuP exciplex species. Complexes with DNA(RNA)-modeling single-stranded homo-oligo/polynucleotides have been studied, since, on one hand, homo-oligo/polynucleotides may provide a regular polymer structure convenient for CuP binding and, on the other, they are free from complications arising from the presence of two different bases as in double-stranded helices.

* To whom correspondence should be addressed. Telephone: (+420) 221 911 471. Fax: (+420) 224 922 797. E-mail: mojzes@karlov.mff.cuni.cz.
† Charles University.
‡ National Academy of Sciences of Belarus. E-mail: sergei.kruglik@polytechnique.fr.
§ Université Pierre et Marie Curie. E-mail: turpin@lpbc.jussieu.fr.

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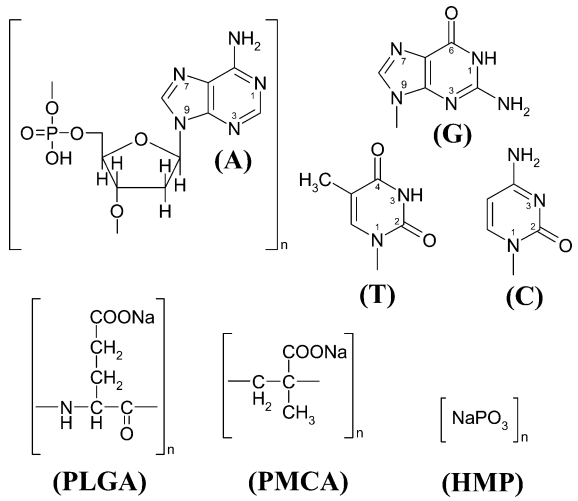

Figure 1. Structure of the single-stranded polydeoxyribonucleotide repeating unit with adenine (A), guanine (G), thymine (T), and cytosine (C) bases. Repeating units of sodium salts of poly(L-glutamic) acid (PLGA), poly(methacrylic) acid (PMCA), and hexametaphosphate (HMP).

It must be noted that, in using nanosecond laser pulses, our study has been focused on a sufficiently long-lived exciplex species having a (sub)nanosecond lifetime. It is important to understand that when very weak exciplex features are detected in transient nanosecond Raman spectra, we cannot reliably judge whether the yield of the exciplex is very low or its lifetime is very short (or both); such questions are not addressed in this paper.

Experimental Section

The copper(II) derivative of 5,10,15,20-tetrakis[4-(*N*-methylpyridyl)]porphyrin (CuP) was prepared from H_2TMPyP4 tetra-*p*-tosylate salt (Aldrich) according to published procedures[21,22] and converted to its chloride form. Sodium salts of single-stranded polynucleotides (poly(rA), poly(dA), poly(dT), poly(rC), poly(rG)), double-stranded polynucleotides (poly(dA-dT)$_2$, poly(dG-dC)$_2$), calf thymus DNA (CT-DNA), and polyanionic poly(L-glutamic) acid (PLGA), poly(methacrylic) acid (PMCA), and hexametaphosphate (HMP) (the last three depicted in Figure 1) were purchased from Sigma and used as received. Oligonucleotides (oligo(dA)$_9$, oligo(dA)$_{30}$, oligo(dC)$_9$, oligo(dT)$_{12}$, oligo(dG)$_{30}$) were synthesized and purified by HPLC in the Laboratory of Plant Molecular Physiology at Masaryk University (Brno, Czech Republic). In the present study, some oligonucleotides of sufficient length ($n \geq 9$) were used instead of the corresponding polynucleotides because of better availability.

All of the complexes studied were prepared as aqueous solutions (20 mM phosphate buffer, pH $\sim$ 7) with an identical total CuP concentration of $\sim 3 \times 10^{-5}$ M and the required nucleic acid-to-porphyrin ratio *R*. Actual concentrations of CuP and nucleic acids were determined spectrophotometrically. Na_2SO_4 (0.2 M; ionic strength $\mu = 0.6$ M) was added to samples when necessary, and the SO_4^{2-} Raman band at $\sim$982 cm^{-1} was used as an internal Raman intensity standard.

Exciplex formation was monitored by pump−probe nanosecond pulsed laser excited RRS (nsRRS) ($\lambda_{exc} = 425$ nm, 20-ns pulse duration, 10 Hz repetition rate) by using the experimental setup described previously.[12,14] The wavenumber scale was calibrated by using toluene. Since relative intensities of nsRRS exciplex features strongly depend on the laser pulse intensity,[14] all spectra were acquired under the same experimental conditions.

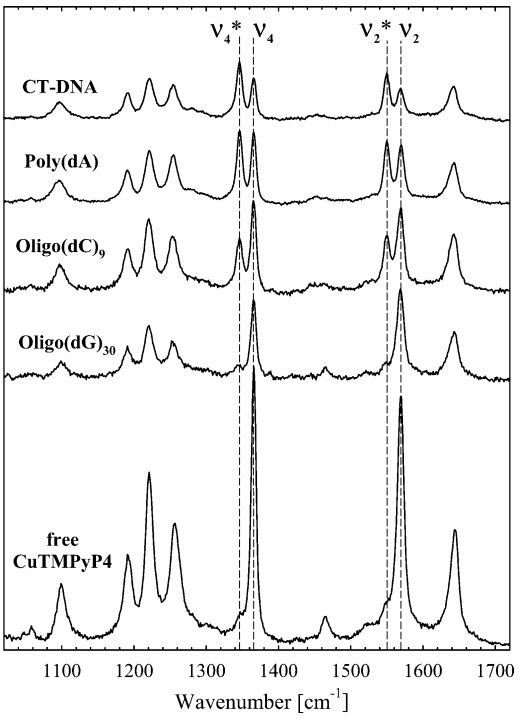

Figure 2. Normalized high-power nsRRS spectra of CuP bound to calf-thymus DNA, DNA-model single-stranded poly(dA), oligo(dC)$_9$, and oligo(dG)$_{30}$, compared with that of free CuP taken under the same experimental conditions.

Results and Discussion

As shown in Figure 2, normalized transient nsRRS spectra of CuP complexed with poly(dA) ($R \sim 80$) and oligo(dC)$_9$ ($R \sim 20$) exhibit exciplex features (ν_2*, ν_4*) of considerable intensities, comparable with those of exciplexes formed under the same experimental conditions in complexes with CT-DNA ($R \sim 30$; Figure 2), poly(dT), or poly(rU) (reported previously[12]). On the other hand, exciplex features observed for complexes with oligo(dG)$_{30}$ ($R \sim 30$) are very weak (Figure 2), that is, of similar intensity to that for those formed with polyribonucleic poly(rA), poly(rC), and poly(rG) (data not shown), confirming previously reported findings about exciplex lack in CuP complexes with single-stranded ribonucleic homopolymers, except poly(rU).[12] Within the accuracy of our nsRRS measurements, exciplexes formed with poly(dA) and oligo(dC)$_9$ exhibit Raman markers at exactly the same wavenumber positions as those with CT-DNA, poly(dT), poly(rU), or water.[14]

As the high yield of exciplex was found for three deoxyribonucleic homopolynucleotides (with A, T, and C) out of four, in contrast to only one ribonucleic (poly(rU)) out of four, structural features of single-stranded deoxyribonucleic acids involve porphyrin binding modes more favorable for exciplex formation than those involved in their ribonucleic pendants. Actually, differences between porphyrin interactions with poly(rA) and poly(dA) reported previously[3,5] were interpreted as resulting from their A- and B-like conformations, respectively.[5] An A-form poly(rA) single-helix was suggested to allow closer and stronger contacts between adjacent π-electron systems and, thus, to promote porphyrin (pseudo)intercalation.[5] Considerable bathochromic shift and substantial hypochromism of the Soret band for poly(rA) (Table 1) are consistent with CuP intercalation, whereas the small bathochromic shift and weak hypochromism observed for poly(dA) support some kind of external binding. Spectral properties of exciplex-giving porphyrin com-

TABLE 1: UV-Vis Absorption Properties of CuP Complexed with Polynucleotides and Polyanions[a]

	$\Delta\lambda_{max}$ (nm)	%H	Δ(fwhm) (nm)
poly(rA)	10.3	28.6	1.7
poly(dA)	2.5	7.5	−4.7
poly(rG)	14.0	38.0	6.8
oligo(dG)$_{30}$	7.6	31.3	7.0
poly(rC)	4.5	29.4	0.3
oligo(dC)$_9$	5.0	11.3	−4.0
oligo(dT)$_{12}$	4.4	−2.7	−5.4
poly(dA-dT)·poly(dA-dT)	3.0	−2.1	−6.0
poly(dG-dC)·poly(dG-dC)	16.0	38.1	2.2
PLGA ($R \sim 3000$)	2.0	1.8	0.2
PMCA ($R \sim 2000$)	−2.0	25.8	5.3
HMP ($R \sim 5000$)	−1.7	18.6	3.6

[a] $\Delta\lambda_{max}$, %H,[21] and Δ(fwhm) represent the shift, hypochromism/hyperchromism (±), and change of the full width at half-maximum of the Soret band, respectively, related to free CuP (λ_{max} = 424.5 nm, fwhm = 27.9 nm). R = [polyanion repeating unit]/[CuP].

plexes (Table 1) show that, besides weak hypo- or hyperchromism and a relatively small bathochromic shift, all of them are characterized by a considerable narrowing of their Soret bands (Δ(fwhm) ∼ −5 nm), in contrast with unchanged or even broadened Soret bands of complexes unfavorable for exciplex formation. Thus, regardless of fine details, one can generally conclude that (pseudo)intercalation into single-stranded helices hinders exciplex formation, whereas some kind of outside binding provides geometries favorable for axial coordination.

Poly(dA) exclusively built with adenine residues having no exocyclic C═O group (Figure 1) can form an exciplex with a considerable efficiency: this raises the question of the nature of the CuP axial ligand. As regards sites for transition metal binding in adenine residues,[23] the following possibilities exist: (a) heterocyclic nitrogens N_1, N_3, and N_7 are good candidates, since they carry lone electron pairs; (b) the base amino substituent is a much less probable candidate because it is not involved in direct metal ion coordination, since the N lone electron pair is delocalized over the π-bonding system of the heterocycle and is not available for metal ion binding; (c) negatively charged phosphate oxygen atoms are improbable ligands, since a comparable yield of exciplex formation was observed for complexes with deoxythymidine (dT) and 5′-dTMP at high nucleoside/nucleotide load (R > 3000);[15] (d) ribose/deoxyribose hydroxyls are known to interact preferentially with alkaline and alkaline earth cations but not with transition metals;[23] (e) the oxygen $O_{4'}$ of the furanose ring can also be considered because of structurally related tetrahydrofuran, that is, oxygen-containing solvent participating in CuTPP exciplex formation;[16,17] nevertheless, due to its weak electronegativity,[23] coordination to the furanose $O_{4'}$ is very unlikely.

As an alternative, coordination of water molecules, trapped in the hydration shell of the polynucleotide, to the central Cu ion can also be suggested to explain the appearance of exciplex features in adducts with poly(dA). Indeed, taking into account immobilization of the porphyrin tightly fixed to the polynucleotide surface, one could speculate that such an exciplex has a considerably longer lifetime than that of one formed in mere aqueous solutions between free CuP and a highly mobile bulk water molecule (<10 ps in the latter case[14]). Lengthening of the exciplex lifetime should result in more intense exciplex features in transient RRS spectra excited with 20 ns laser pulses. In principle, any hydrated polymer consisting of repeating, negatively charged units, to which CuP binds by a nonintercalative mode, would provide a complex geometry favorable for exciplex formation with immobilized water as axial ligand. To

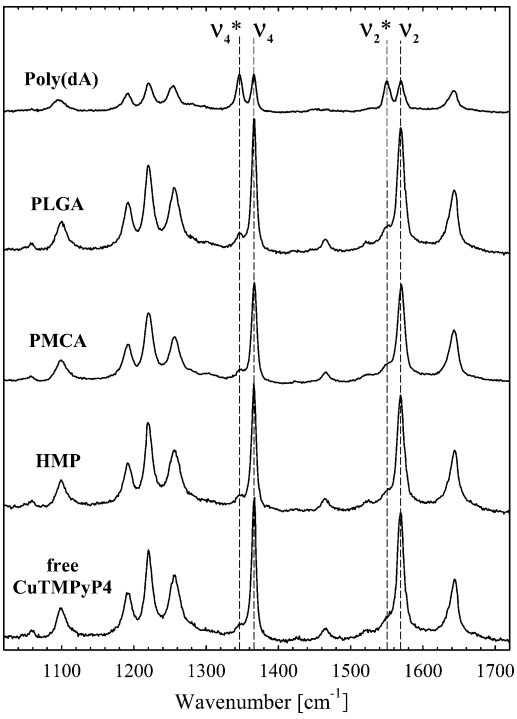

Figure 3. Normalized high-power nsRRS spectra of CuP mixed with poly(dA), PLGA, PMCA, and HMP, compared with that of free CuP taken under the same experimental conditions.

test this possibility, CuP adducts with anionic polyelectrolytes PLGA, PMCA, and HMP have been investigated under high-power irradiation. It is worth noting that PLGA has been reported to form adducts with cationic (metallo)porphyrins, exhibiting similar spectroscopic features to those of porphyrin complexes with poly(dA).[3,4] Since, in contrast to polynucleotides, these polyanions possess no aromatic rings, the intercalative binding mode can be excluded. Moreover, to avoid formation of porphyrin−porphyrin assemblies along the polyanionic chain as a template, a high polymer load ($R \sim 2000-5000$) was used. Thus, regardless of structural details, the central atom of the CuP complexed with hydrated PLGA, PMCA, and HMP could be exposed to water molecules from the hydration shell of the polymer. It should be noted that, because CuP complexes formed with polyanions are not as stable as those formed with nucleic acids (especially at high ionic strength), no Na_2SO_4 salt was added into the samples. As seen from Figure 3, none of the polyanions tested provides exciplex features notably different from those of free CuP in bulk water. Therefore, neither immobilized water nor any functional group present in polyanions (dissociated carboxyl COO^-, C═O of amide groups, dissociated PO_3^-) can serve as an efficient axial ligand for a long-lived exciplex.

Consequently, heterocyclic nitrogens N_1, N_3, or N_7 from adenine base acting as axial ligands seem to provide the most plausible explanation for exciplex features' creation in complexes with poly(dA). It is worth mentioning that the hypothesis of base nitrogen participation in exciplex formation for CuP complexes with nucleic acids has already been proposed previously.[24] To explain their 70-ps transient Raman spectra, Jeoung et al.[24] suggested formation of an exciplex between CuP and poly(dG-dC)$_2$, with cytosine N_3 as an axial ligand. However, a later 2-ps TR^3 study[14] excluded exciplex formation for the CuP complex with poly(dG-dC)$_2$. To the best of our knowledge, the current results represent the first experimental evidence for exciplex formation with a nucleobase nitrogen as an axial ligand, observed for CuP complexes with nucleic acids.

Conclusion

Results presented here lead us to suggest that, besides thymine and uracil previously known to participate in exciplex formation, also *adenine* and *cytosine* can serve as axial ligands for photoexcited CuP, in complexes with some single-stranded deoxyribopolynucleotides (e.g. poly(dA), poly(dC)). The case of adenine is especially interesting, since it possesses no exocyclic C═O group. Basic endocyclic nitrogens (N_1, N_3, or N_7) of adenine are suggested to serve as electron-density donors to coordination bond with the porphyrin central copper ion, giving rise to a third exciplex species called CuP*N. Our study confirms that the porphyrin binding mode mediated by structural and conformational properties of the polynucleotide plays a determinant role in exciplex formation, since different exciplex yields are found for ribo- and deoxyribo-, single-stranded homopolynucleotides consisting of the same nucleobases (poly(rA) and poly(rC) vs poly(dA) and poly(dC), respectively), and almost no exciplex is found with poly(dG) and poly(rG), despite guanine electron-donating groups (C_6═O, N_3, or N_7) that could serve as axial ligands. Explanation of strong exciplex features as arising from the coordination of immobilized water molecules from the polymer hydration shell is improbable, since no exciplex was found for the complexes formed with a series of hydrated polyanions.

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