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Photoinduced Species of Cationic CoII Porphyrin in Complexes with AT−Containing Nucleic Acids Characterized by Resonance Raman and Transient Absorption Spectroscopies

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A study of the water-soluble cationic CoII-tetrakis(N-methyl-4-pyridiniumyl)porphyrin, CoIITMpyP(4), complexed with calf thymus DNA and synthetic double-stranded and single-stranded adenine and thymine containing polynucleotides, was carried out by resonance Raman (RR) and transient absorption spectroscopies. Under high intensity nanosecond pulsed excitation, transient ν_4* and ν_2* bands appear in the RR spectra of CoIITMpyP(4) in the presence of DNA, poly(dA-dT)$_2$ and poly(dT). On the basis of picosecond transient absorption measurements the lifetime of the corresponding transient species, ascribed to CoIITMpyP(4) electronic excited states of charge transfer (CT) origin (π, dz^2), was found to be 10 ± 4 ns, i.e. three orders of magnitude longer than the excited state lifetime of free CoII porphyrin in aqueous solution. Such a considerable lengthening of the CT state is interpreted in terms of electron transfer from the pyridyl groups to the core ring, owing to the rotation of these groups and subsequent molecular flattening necessary to accommodate the porphyrin within the minor groove nearby T and AT sites.

Keywords: Cationic CoII porphyrin, polynucleotides, resonance Raman spectroscopy, transient absorption, exited state.

Introduction

Porphyrin complexes with nucleic acids (NA) have been extensively investigated during the last three decades to exploit their medical and biological applications.[1-8] In this relation, metal derivatives of water-soluble porphyrins are of interest in many areas as probes of the DNA structure,[9,10] as DNA breaks,[11] for enhancement of restriction enzyme activity,[12] as photoactive insecticide,[13] and as possible photosensitizers to be used in photodynamic therapy.[6]

Transition metal complexes of water-soluble cationic porphyrins and their interactions with NA attract special attention. Though they have no long-lived excited states and do not show any photodynamic activity, these compounds may be useful as drugs for anticancer treatment since they are able to induce strand breaks in DNA in the presence of a variety of oxidizing agents.[14-16] Moreover, transition metal porphyrins such as NiII and CuII complexes show various binding modes to nucleic acids mediated by axial ligand uptake/release processes, that depend on solution properties and/or photoexcitation conditions.[17-20] Thus their investigation photoinduced processes can help to probe local DNA structures and dynamics.

A number of questions concerning the metalloporphyrin - NA interactions remain to be not clearly understood, in particularly with respect to the effects of DNA-binding on its morphology changes and to the porphyrins photophysical properties. In this regard, resonance Raman spectroscopic techniques (RRS) used together with time-resolved absorption (TA) spectroscopy has been proved can give valuable information.[20,21]

In this study, we used the above mentioned two spectroscopic techniques for investigation of the photoinduced processes for CoII derivative of the water-soluble cationic tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin (CoIITMpyP(4), Figure 1) in its complex with DNA as well as poly(dA-dT)$_2$ and poly(dT). Potential applications of CoII porphyrins are related to their catalytic activity in oxidation/reduction reactions, via metal axial ligand exchanges. They are also known to be growth inhibitors of human malignant melanoma cells.[22]

We have earlier reported the study of steady-state interaction of CoIITMpyP(4) with DNA and model polynucleotides.[22] Porphyrin binding modes to a set of AT- and GC-containing NA have been proposed. Here we report the study of the excited CoIITMpyP(4) complexes with AT-containing NA by using resonance Raman and TA
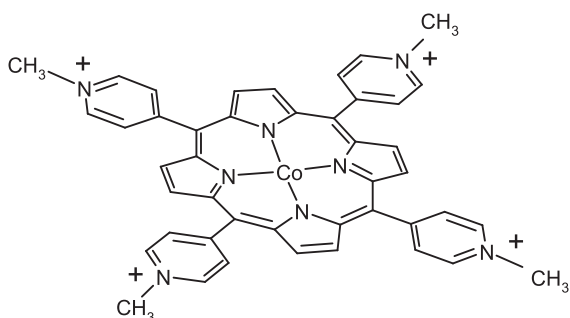

Figure 1. Structure of CoIITMpyP(4).

techniques. The current Raman data for photoexcited Co^{II} porphyrin were analyzed by using the results of the work [22] and of our earlier RR data acquired for free Co^{II}TMpyP(4) solutions.[23]
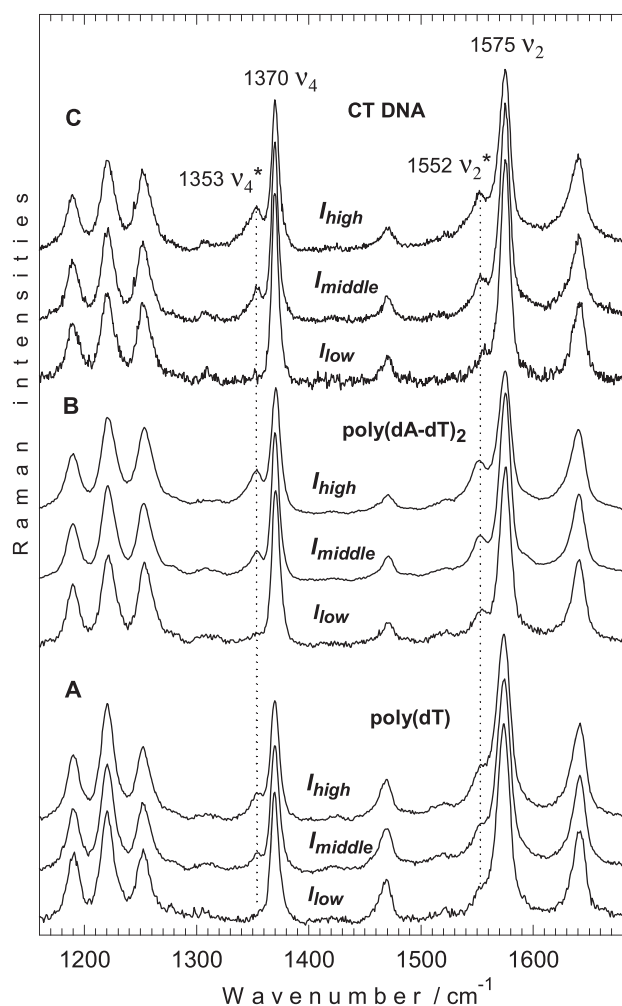

Figure 2. Excitation power dependent resonance Raman spectra of Co^{II}TMpyP(4) bound to poly(dT) (A), poly(dA-dT)$_2$ (B), DNA (C). Incident power density of 425 nm laser pulsed irradiation increases from $I_{low} \sim 1 \cdot 10^5$ W/cm^2 to $I_{high} \sim 4 \cdot 10^7$ W/cm^2.

Experimental

Cobalt(II)- and cobalt(III)-tetrakis(*N*-methyl-4-pyridiniumyl) porphyrins (tetraiodide and pentaiodide salts, respectively) were obtained by Malinovskii V.L. as described in the literature.[24] Calf thymus DNA, poly(dA-dT)$_2$, poly(dT) were purchased from Sigma Chemical Co, and used without further purification. Since free Co^{II} porphyrin in mere phosphate buffer solution (PBS) can rapidly be oxidized, to obtain Co^{II} porphyrin-nucleic acid solutions with the lowest possible contribution of the Co^{III} species, required amounts of Co^{II}TMpyP(4) powder was added directly to the nucleic acids solutions in a 25 mM PBS at ionic strength μ=0.03 M (p*H* 6.8). Base pair/porphyrin molar ratio (R) was *ca*. 30.

Stationary absorption spectra were obtained on Beckman UV5270 and Cary-1E UV-visible spectrophotometers. All measurements were carried out in 10×10 or 10×4 mm quartz and plastic cells at room temperature.

RR spectra were recorded with a 90^0 scattering geometry on a T64000 Jobin-Yvon Raman spectrometer equipped with a CCD detector cooled up to 140 K by liquid nitrogen. Pulsed excitation at 422-440 nm (repetition rate 10 Hz, pulse duration 20 ns) was obtained

by frequency mixing the laser emission of the LD 700 dye with the 1064 nm of the Nd^{3+}-YAG laser in a nonlinear crystal. Samples were contained in stirring standard 10 mm spectrofluorometric quartz cells. Typical experimental conditions were 3-5 cm^{-1} spectral slit width, 0.8 cm^{-1} increment, 20-30 accumulations of 20-s time of integration. The wavenumber peak positions were accurate within ±1.0 cm^{-1}.

To obtain transient absorption data sample solutions were excited with the second harmonic of a passive mode-locked Nd - YAL laser (λ_{exc} = 540 nm, pulse width ~ 12 ps), *i.e.* in the *Q*-band of the porphyrins. An optical parametric oscillator tunable over the Soret absorption band was used to probe the samples. All TA experiments were carried out in spinning quartz cells of 1 mm optical pathlength.

Results and Discussion

Co^{II}TMpyP(4) is easily oxidized by molecular oxygen dissolved in solutions. From our observations reported earlier [22] it was assumed that Co^{II}TMpyP(4) in the presence of DNA, poly(dA-dT)$_2$ and poly(dT) is stable. In contrast, it is rapidly oxidized to Co^{III} species upon complexation with poly(dG-dC)$_2$ and poly(G). The observed effects of Co^{II} stabilization or enhanced oxidation were proposed to be connected with the axial ligation processes which usually occur for transition metal porphyrins upon interactions with NA. A special role of immobilized water bound to Co^{II} as axial ligand was suggested to fulfill conditions which prevent fast Co^{II}TMpyP(4) oxidation when it is complexed with thymine and adenine containing NA.

It is interesting that namely in the case of Co^{II}TMpyP(4) complexes with AT-containing NA new RR bands appear at high power pulsed excitation. Figure 2 shows RR spectra of Co^{II} porphyrin in mixture with DNA, poly(dA-dT)$_2$ and poly(dT) excited with power near 10^8 W/cm^2 (10 ns pulses). In increasing the excitation power, new bands ν_4* and ν_2* appear at 1353 cm^{-1} and 1552 cm^{-1}, *i.e.* downshifted relatively to their stationary ν_4 and ν_2 counterparts by 17 and 23 cm^{-1}, respectively. This is accompanied by a depletion of the stationary species. The corresponding intensities decrease when the excitation power increases, this being completely reversible with the excitation power. The intensities of these additional bands are approximately equal in complexes with DNA and poly(dA-dT)$_2$, but weaker for complex with poly(dT). There is no additional feature at any other wavenumber of the spectra. It is interesting that under similar experimental conditions these additional bands were not observed in the RR spectra for Co^{II}MpyP(4) complexed with poly(dG-dC)$_2$ and in free solution, as well as for Co^{III}MpyP(4) in PBS and with all nucleic acids studied (data not shown).

To explore the origin of the long-lived photoinduced species for Co^{II}TMpyP(4) - NA complexes, photophysics of free Co^{III}- and Co^{II}TMpyP(4) in solution and in complexes with DNA and poly(dA-dT)$_2$ was studied by picosecond transient absorption spectroscopy. Picosecond excitation of Co^{III}TMpyP(4) in the *Q*-band resulted in a very fast appearance of an optical density changes in the Soret region, followed by a very fast (characteristic relaxation time constant τ ≤ 20 ps) relaxation to the initial state. Figure 3 shows the typical fast kinetics obtained for Co^{III}TMpyP(4) complexed with DNA with 10 ± 3 ps decay time. Similar fast reversible kinetics was observed for the free Co^{II} porphyrin species in solution (data not shown). Such fast excited state deactivation

likely implies that only intramolecular relaxation processes, *i.e.* not concerned with axial ligand release and back binding processes, occur in all cases. Indeed, it is well documented that for four-coordinated Co^{II} porphyrins, for which axial deligation and back recombination processes are impossible, all intramolecular relaxation processes are completed within 50 ps after excitation.[25-27] On the other hand, if Co porphyrins are five- or six-coordinate, a photoinduced axial ligand release can occur with following ligand back recombination. In this case the latter process is relatively slow with time constants in the time scale of hundreds of picoseconds or longer, depending on the ligand concentration.[25,26,28]
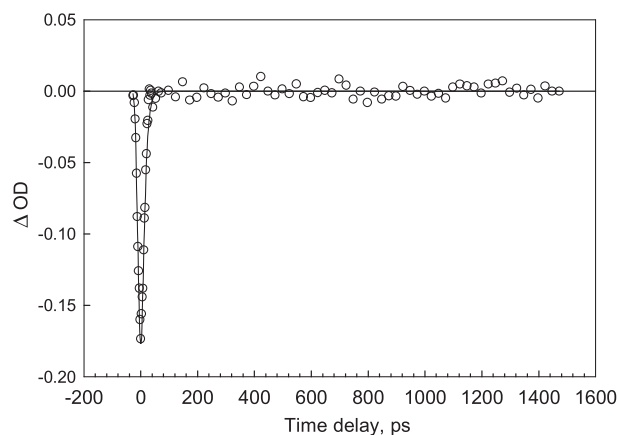

Figure 3. Transient absorption decay of Co^{III}TMpyP(4) - DNA complex with excitation at 540 nm and probe at 434 nm. Solid line curve represents a least-square fit to a single-exponential function with 10±3 ps time constant.

However, Co^{II}TMpyP(4) in complexes with DNA and poly(dA-dT)$_2$ was found to exhibit an additional long-lived relaxation component. Figure 4 shows the typical kinetics obtained for the Co^{II}TMpyP(4)-poly(dA-dT)$_2$ complex.
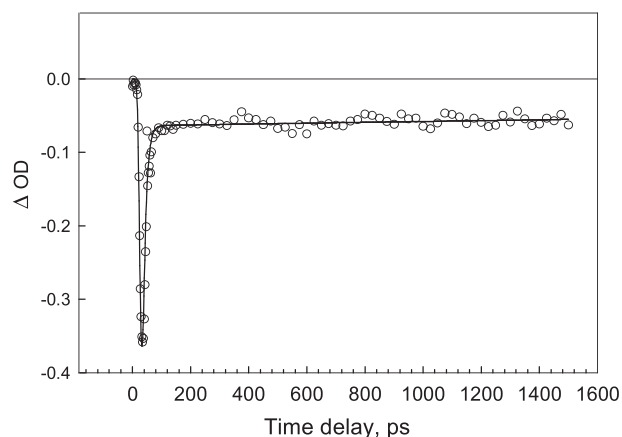

Figure 4. Transient absorption decay for Co^{II}TMpyP(4) in poly(dA-dT)$_2$ with excitation at 540 nm and probe at 425 nm. Solid line curve represents a least-square fit to a double-exponential function. Fitted decay times are: 10 ± 3 ps (fast component) and 10 ± 4 ns (slow component).

Relaxation time constant of the short-lived signal was evaluated as τ_1 =10 ± 3 ps, whereas that of the long-lived signal was found to be as long as τ_2 = 10 ± 4 ns. Double-exponential kinetics with similar relaxation time constants

(but with much less intense long-lived signal) were also found for Co^{II}TMpyP(4) complexed with DNA.

The appearance of this long-lived photoinduced species well correlates with the observation of additional RR bands reported above. A correspondence between picosecond TA results and RR data obtained under high power pulsed excitation of Co^{II}TMpyP(4) complexes with AT containing polynucleotides (DNA, poly(dA-dT)$_2$, poly(dT)) enables us to conclude that the 10 ns transient absorption signal (Figure 4) and the additional RR bands (Figure 2) are manifestations of the same transient species. Indeed, 20 ns pulses of laser excitation used in our RR saturation experiments were obviously able to populate noticeably transient species having nanosecond lifetimes, but not species whose lifetimes are tens of picoseconds and shorter.

Since all of the available data indicate that Co porphyrin excited state lifetimes are shorter than 50 ps,[25-27] it would be natural to suggest that the 10 ns component observed for Co^{II}TMpyP(4)-NA complexes belongs to a relatively slow process of axial ligand back recombination to the porphyrin ground state after ultrafast photoinduced release of the ligand, as observed earlier for various Co porphyrins bearing axial ligands.[25,26,28]

However, our RRS data are in strong contradiction with this suggestion. We are convinced that a change of the number of Co axial ligands due to a photoinduced ligand ejection could not result in such very large shifts ($\Delta\nu_4$ = 17 cm^{-1}, $\Delta\nu_2$ = 23 cm^{-1}) for RR bands corresponding to macrocycle vibrations (see for comparison axial ligand exchange effects on RR spectra of Co^{II}TMpyP(4) in our recent work[23]). Only considerable perturbation of the porphyrin π system can lead to such large wavenumbers shifts for skeletal vibrations. We suggest, therefore, that the long-lived photoinduced species observed for Co^{II}TMpyP(4) complexed with AT containing NA belong to an excited state, *e.g.* Co^{II} porphyrin CT state of (π,d_z^2) nature.

Under this hypothesis the porphyrin macrocycle perturbation can be achieved owing to an enhanced d_π-π* -back-donation effect upon electron transfer to d_z^2 orbital. This results in antibonding e_g* orbital population and, therefore, macrocycle bonds weakening. In principle, d_z^2 orbital population gives rise to an energy increase (destabilization) not only for this orbital, but simultaneously for d_π orbitals, that is accompanied by a more effective d_π - e_g* overlapping. Such a mechanism has been suggested to occur in the case of chemically generated monoanion of Co^{II}OEP, when an additional electron was added to d_z^2 orbital.[28] In this situation shifts of structure sensitive RR bands were as large as those observed here for photoexcited complexes of Co^{II}TMpyP(4) with NA.

A question now arises: what is the reason of such a lengthening, in comparison with aqueous solution, of the Co^{II} porphyrin CT state lifetime when the porphyrin is bound to nucleic acids? The above CT state population corresponds to the porphyrin cation radical and Co^I formation. Thus, the CT state deactivation dynamics should be influenced by the extent of coupling between the π system and the cobalt ion. The significantly slower decay of the CT state for Co porphyrin bound to NA *vs* free in solution can be assigned to an increased electron transfer from the pyridyl moiety to the porphyrin ring, owing to a decrease of the pyridyl ring-

to-porphyrin dihedral angle. We believe that such a flattening of the porphyrin structure is favourable for its disposition within narrow DNA grooves, especially minor groove (such kind of binding mode was proposed for Co^{II}TMpyP(4)-DNA complex.[22]) Moreover, electronic structure and photophysics of namely cationic tetramethylpyridyl-substituted porphyrins can be strongly influenced by such structure dependent charge transfer processes since they possess a large charge located on the pyridyl rings, whereas the porphyrin core is scarce with electron density. Earlier similar conjugation-dependent decrease of the rate of CT state decay has been observed for tetraruthenated cobalt porphyrins.[29] It is worth to note that, although CT states of metalloporphyrins routinely yield picosecond lifetimes, there is an example of ruthenium(II) porphyrins for which CT states were observed with lifetimes of tens of nanoseconds.[30]

Conclusions

From resonance Raman data and transient absorption spectra kinetics we conclude that photoinduced species observed for Co^{II}TMpyP(4) bound with DNA and AT-containing nucleic acids are related to population of a long-lived (10 ± 4 ns) electronic excited state of charge transfer (CT) origin ((π, d_z^2)). Such a considerable lengthening of the CT state (three orders of magnitude longer than the excited state lifetime of free Co^{II} porphyrin) is interpreted in terms of electron transfer from the pyridyl groups to the core ring, owing to the molecular flattening necessary to accommodate the porphyrin within the minor groove nearby AT sites.

It should be noted the similarity of RR spectra changes at high power nanosecond excitation as well as TA data observed for Co^{II}TMpyP(4) and for exciplex between excited Cu^{II} porphyrins and thymine-containing nucleic acids.[20,21] The formation of long-lived exciplex in the case of Cu^{II}TMpyP(4) was connected the axial ligand uptake/release processes. Clarification of generality in the photoinduced processes for both Co^{II-} and Co^{II} porphyrin complexes with AT-containing NA needs further development.

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