A Resonance Light-Scattering Analysis of the Suprahelical Helixes of Nucleic Acids Induced by 5,10,15,20-Tetrakis[4-(trimethyammonio)phenyl]porphine

Cheng Zhi Huang,# Ke An Li* and Shen Yang Tong

Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, the People's Republic of China

(Received October 7, 1996)

This paper discusses the usefulness of resonance light-scattering spectroscopy (RLSS), which can be obtained by using an ordinary spectrofluorimeter, for the analysis of the suprahelical helixes of nucleic acids induced by 5,10,15,20-tetrakis[4-(trimethyammonio)phenyl]porphine (H_2tapp). Depending on the acidity of the aqueous solution, the titled porphyrin has two species, $[H_2tapp]^{4+}$ and $[H_4tapp]^{6+}$. Both species can stack on appropriate conditions along the surface of nucleic acids in the mode of long-range assembly; and this leads to the formation of suprahelical helixes of nucleic acids. Enhanced resonance light-scattering signals can be observed for the porphyrin-induced suprahelical helixes of nucleic acids with the maximal resonance light scattering near to 432 nm for $[H_2tapp]^{4+}$ and near to 452 nm for $[H_4tapp]^{6+}$. The exciton splitting signals, which are generally associated with the aggregation of porphyrins, as described by molecular-exciton theory, are observed at 428 nm in the RLS spectra, analogous to the circular dichroism spectra when the molar ratio of $[H_2tapp]^{4+}$ to nucleic acids (R) is greater than 0.89. These results indicate that porphyrin–porphyrin interactions occur in the suprahelical helixes of nucleic acids.

Whether considering chemistry or biochemistry, porphyrins are important and interesting compounds owing to their extensively electron-delocalized structure in the nucleus and their biological activities. Although the initial interest in porphyrin–nucleic acid interactions arose in conjunction with photodynamic therapy,[1] greater interest in the interactions stems from the porphyrin antiviral (including HIV-1)[2] and anti-cancer activities.[3] Since porphyrins can serve as useful probes of the structures and functions of nucleic acids,[4—12] and studies on water-soluble porphyrins with biologically active macromolecules can supply a good model for studying the complex biophysical process, the interactions of porphyrins with nucleic acids have raised a great interest among chemists and biochemists during the recent decades.

Since Fiel's first article about the interactions of porphyrins with nucleic acids,[1] investigations concerning the interactions have mainly focused on the 5,10,15,20-tetrakis(1-methyl-4-pyridinio) families of metallo and nonmetallo porphyrins.[13] The possible reasons that the studies concentrated on these families are the positively charged substituents, a lower aggregation trend of the (1-methyl-4-pyridinio) groups, and the easy preparation of metalloporphyrins.[14] There are three binding modes pertaining to the interactions of porphyrins with nucleic acids, depending on the porphyrins' molecular symmetry; the number and position of the positive charge of porphyrins; the interaction conditions, such as pH and ionic strength of the solutions; and the molar ratio of porphyrins to nucleic acids (R).[15] Generally, when R and the ionic strength are low, those porphyrins with axial ligands, such as metalloporphyrins of Fe(III),[4] Co(II),[5] Mn(III),[6] Zn(II)[7] of the 5,10,15,20-tetrakis(1-methyl-4-pyridinio) families, bind nucleic acids in a groove mode; however, those without axial ligands, such as the nonmetallo and their metalloporphyrins of Cu(II),[8] Ni(II),[9,10] Pt(II),[9,10] Au(III),[11] V(II),[12] of the 5,10,15,20-tetrakis(1-methyl-4-pyridinio) families, bind with nucleic acids in an intercalating mode. An exception to the interactions is the free-base porphyrin, 5,10,15,10-tetrakis[4-(trimethyammonio)phenyl]porphine ($[H_2tapp]^{4+}$; its molecular structure is shown in Fig. 1), with nucleic acids. $[H_2tapp]^{4+}$, stacking together along the surface of the nucleic acid molecule,[16,17] does not bind with nucleic acids in the groove or intercalating modes. If the molar ratio (R) is sufficiently large, the stacking of $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ leads to the formation of suprahelical helix structures of nucleic acids.[16,17]

Resonance light-scattering spectroscopy, obtained by using an ordinary spectrofluorimeter,[14] is sensitive and informative in chemistry and biochemistry research. Analogous to resonance-enhanced Raman scattering for aggregated molecules in aqueous solutions,[18,19] the basic theory of the resonance light-scattering spectroscopy is the same as that of the resonance-enhanced Rayleigh scattering in an absorption medium in which aggregated particles can exist.[14,20—28] We found that strong resonance-enhanced Rayleigh scattering

Institute of Environmental Chemistry, Southwest Normal University, Chongqing 630715, the People's Republic of China.

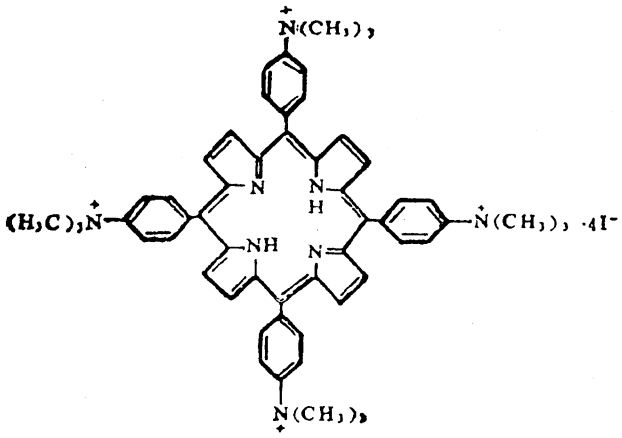

Fig. 1. Molecular structure of 5, 10, 15, 20- tetrakis[4-(trimethyammonio)phenyl]porphine ($[H_2tapp]^{4+}$).

is produced when the molar ratio of the titled porphyrin to nucleic acids is high in an aqueous solution with low ionic strength, in such a case that suprahelical helixes of nucleic acids can be formed.[16,17] Accordingly, the observation of a resonance light-scattering enhancement can act as an indication of the formation of the suprahelical helixes of nucleic acids. We have reported on a sensitive determination of nucleic acids in synthetic samples based on the enhancement effect of nucleic acids on resonance light-scattering intensity of dyes.[26,27] In the present paper we discuss the features of the resonance light-scattering spectra in terms of the formation of suprahelical helixes of nucleic acids in the presence of $[H_2tapp]^{4+}$ and $[H_4tapp]^{6+}$.

Experimental

Apparatus. All of the spectra and intensities of resonance light scattering were obtained using a Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan) with a quartz cell (1×1 cm). A Shimadzu UV 265 spectrophotometer (Kyoto, Japan) was used to obtain the absorption spectra. A WH-861 vortex mixer (Jiangsu Instruments, Inc., China) was used to blend a mixture in a volumetric flask, and an 821 pH meter (Zhongshan University, China) was used to measure the pH of a solution.

Reagents. Stock solutions of DNAs and RNA were prepared by dissolving commercial calf thymus DNA (CTDNA, Beitai Biochemical Co., Chinese Academy of Sciences, Beijing, China), fish sperm DNA and yeast RNA (FSDNA and YRNA, Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, China) in doubly deionized water at 4 °C. It took 24 h or more to dissolve DNAs as an aqueous solution by occasionally gentle shaking. The concentrations of nucleic acids were calculated according to the absorbance at 260 nm by using the molar absorptivity of the nucleic acids: ε_{DNA} (6600 M^{-1} cm^{-1}) and ε_{RNA} (7800 M^{-1} cm^{-1}).[29] Working solutions of the nucleic acids were prepared in the following concentration series (×10^{-5} M): 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, 3.0, 3.3, 3.6 and 7.5 (1 M = 1 mol dm^{-3}).

The free-base porphyrin, $[H_2tapp]^{4+}$, was synthesized according to the literature[30] and identified by ^{1}H NMR and IR. This compound was dissolved in doubly deionized water. The concentration of the solution was determined according to the absorbance at 412.0 nm (the Soret maxima) by using $\varepsilon = 4.16\times10^5$ M^{-1} cm^{-1} at pH 7.48 (I = 0.004).[30]

Tris-HCl buffer was prepared by dissolving 6.1 g of tris(hydroxylmethyl)methanamine in 250 ml water, adding 400 ml of 0.1 M HCl and then diluting to 1000 ml with water. The pH of the buffer solution was 7.48.

All of the reagents were of analytical reagent grade and used without further purification. The water used throughout was doubly deionized.

Standard Procedures. After 0.60 ml of a standard nucleic acid solution and 0.60 ml of a $[H_2tapp]^{4+}$ solution were added with different solution affluxes along the wall of a dry 10-ml volumetric flask, the mixture was fully vortexed, and 1.0 ml of the Tris-HCl buffer solution was added. The mixture was then diluted to 10 ml with water, at last, and completely mixed.

The resonance light-scattering spectrum was obtained by synchronously scanning the excitation and emission monochromators of the RF-540 spectrofluorimeter in the wavelength region from 350 to 750 nm with $\Delta\lambda$ = 0.0 nm. The slit width for the excitation and emission was 5.0 nm.

Results and Discussion

Resonance Light-Scattering Spectra. The porphyrin investigated in this study can have two chemical forms because of the protonation of the two pyrrolic nitrogen atoms in the porphyrin macrocycle (pK_{a_1} = 3.95, pK_{a_2} = 4.11, I = 0.1, 25 °C).[30] The two species are $[H_2tapp]^{4+}$ and $[H_4tapp]^{6+}$, and the formation of the species depends on the pH of the aqueous solution. At pH 7.48, $[H_2tapp]^{4+}$ has D_{2h} spectroscopic features and has absorption bands at 412.0, 513.0, 553.0, 579.0, and 631.0 nm, as shown in Fig. 2. These absorption bands originate from the second excited singlet state (the Soret maxima), B(0,0); the lowest-energy excited singlet state, Q(0,0); and the vibronic state, Q(1,0). Both the Q bands split into Q_y(1,0), Q_y(0,0), Q_x(1,0), and Q_x(0,0). At pH 1.86, $[H_2tapp]^{4+}$ is protonated and exists as $[H_4tapp]^{6+}$,

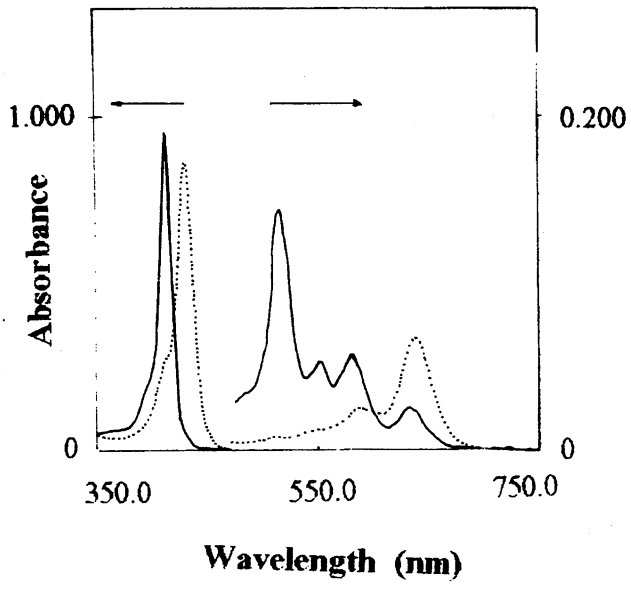

Fig. 2. Absorption spectra of the titled porphyrin at pH 7.48 (solid line) and 1.86 (dotted line). The concentration of the porphyrin, 2.4×10^{-7} M. Ordinate in the wavelength range from 470.0 to 750.0 nm was enlarged by 5.

having D_{4h} spectroscopic features and characterized by no splitting of the Q bands. For $[H_4tapp]^{6+}$, the Soret band is located at 430.8 nm and the Q bands are observed at 590.0 and 639.2 nm. The two Q bands are assigned to Q(1,0) and Q(0,0), respectively.[31] The resonance light-scattering results are consistent with the conclusions derived from the absorption characteristics of the porphyrin species. Figure 3 shows the resonance light scattering spectra of the titled porphyrin in neat water at different pH, together with those of background solutions for a comparison. Regardless of the pH value, the presence of the porphyrin has little influence on the spectra of water and buffer background solutions, except for the Soret maxima, at which photon absorption leads to local minima (at 413.8 nm for $[H_2tapp]^{4+}$ and 430.4 nm for $[H_4tapp]^{6+}$). At slightly longer wavelengths, a characteristic "overshoot" appears compared with the resonance light spectra of water and the buffer background solutions.

The minima of the light-scattering spectra near to the Soret band of $[H_2tapp]^{4+}$ and $[H_4tapp]^{6+}$ results from the self absorption of the two porphyrin species. According to the theory of resonance light scattering, the intensity of resonance light scattering depends on the scattering cross section and absorption cross section of a molecule scatterer.[14,20–24] Even if the molar absorptivity of the molecule is very large, the intensity of resonance light scattering detected by instruments is small, or as even likely to be difficult to detect when the volume of the molecule is very small. However, if the molar absorptiontivity and volume of the molecule are large, an enhancement of the resonance light scattering would be observed. Additionally, different from the resonance light scattering of $[H_2tapp]^{4+}$, $[H_4tapp]^{6+}$ has a scattering peak at 639.2 nm where the Q(0,0) absorption band is located, as shown in Fig. 2. It is likely that the 639.2 nm scattering peak is due to the lowest singlet excited state.

If nucleic acid solutions were added to an $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ solution, a strong enhancement of the resonance light scattering can be observed. Figure 4 shows that the minima of the enhanced light scattering at the Soret band still exist, and that the maximum wavelength of enhanced resonance light scattering is located at the longer wavelength side of the Soret band. The shoulder peaks in the wavelength range of 500–600 nm can be observed, which may be correlated to the Q(1,0) absorption band. In addition, from Fig. 4, it can be seen that the resonance light scattering of $[H_4tapp]^{6+}$ at 639.2 nm, where the Q(0,0) absorption band is located, is also enhanced by nucleic acids.

It is interesting that a small shoulder peak at 405 nm appears (solid line in Fig. 4 and dotted lines in Fig. 5). The small shoulder peak makes a minima at 418.0 nm with the maximal enhanced light-scattering peak. The appearance of the minima, located identically with the exciton splitting, which is generally associated with aggregation, as defined in the molecule exciton theory and is often studied by the CD spectrum,[16,17] is indicative of porphyrin–porphyrin interactions in the porphyrin–nucleic acid interacting system.[32] Figure 5 clearly shows that the exciton splitting takes place upon increasing the concentration of $[H_2tapp]^{4+}$. The minimum at 418.0 nm does not appear until the molar ratio of $[H_2tapp]^{4+}$ to a nucleic acid (R) is greater than 0.89. Thus, the exciton splitting can be observed only if the concentra-

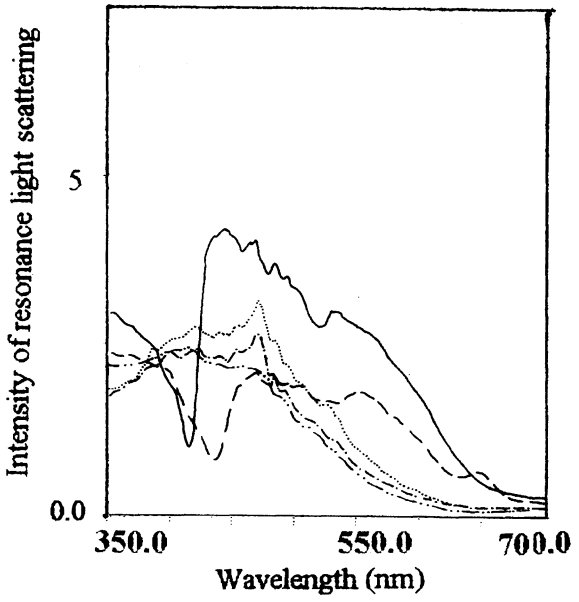

Fig. 3. Resonance light scattering spectra of the titled porphyrine at pH 7.48 and 1.86. Solid line, $[H_2tapp]^{4+}$ (pH 7.48); dashed line, $[H_4tapp]^{6+}$ (pH 1.86); dotted line, blank solution made up of water and Tris-HCl buffer (pH 7.48); dash-dotted line, blank solution made up of HCl solution (pH 1.86), dash-dot-dotted line, doubly deionized water. Concentration of porphyrins, 2.4×10^{-6} M.

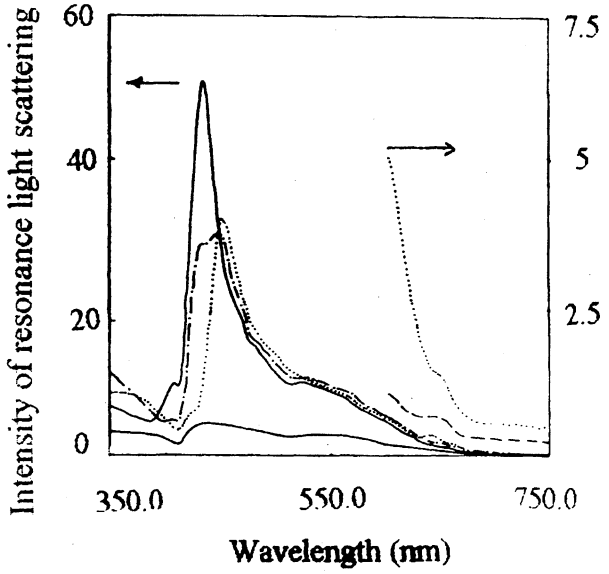

Fig. 4. Enhancement effect of CTDNA on the resonance light-scattering of $[H_2tapp]^{4+}$. Solid line, pH 7.48, the upper, CTDNA + $[H_2tapp]^{4+}$, the lower, $[H_2tapp]^{4+}$; dash-dotted line, pH 3.78, CTDNA + $[H_4tapp]^{6+}$; dotted line, pH 1.86, CTDNA + $[H_4tapp]^{6+}$; dashed line, pH 1.86, $[H_4tapp]^{6+}$. From 600 to 750 nm the ordinates were enlarged by 8 for the two curves of pH 1.86. Concentration; CTDNA, 9.0×10^{-7} M; porphyrins, 1.2×10^{-6} M.

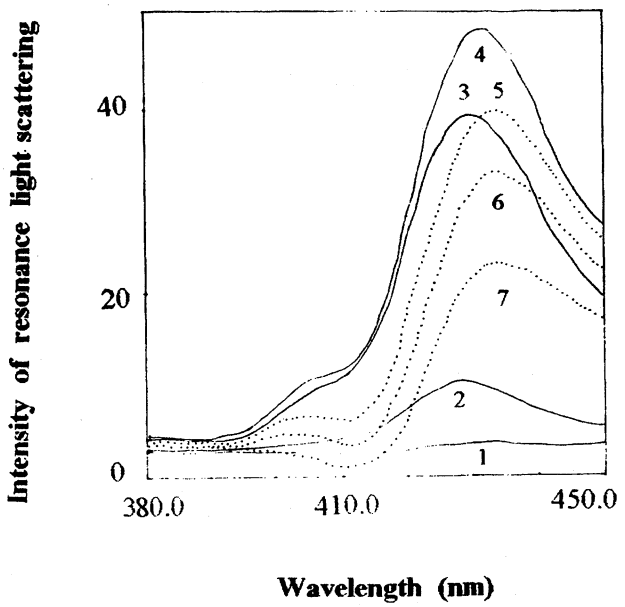

Fig. 5. Exciton splitting features of the resonance light-scattering spectra. pH 7.48; Concentration of CTDNA: 9.0×10^{-7} M. R; 1, 0.22; 2, 0.44; 3, 0.67; 4, 0.89; 5, 1.33; 6, 1.78; 7, 2.67.

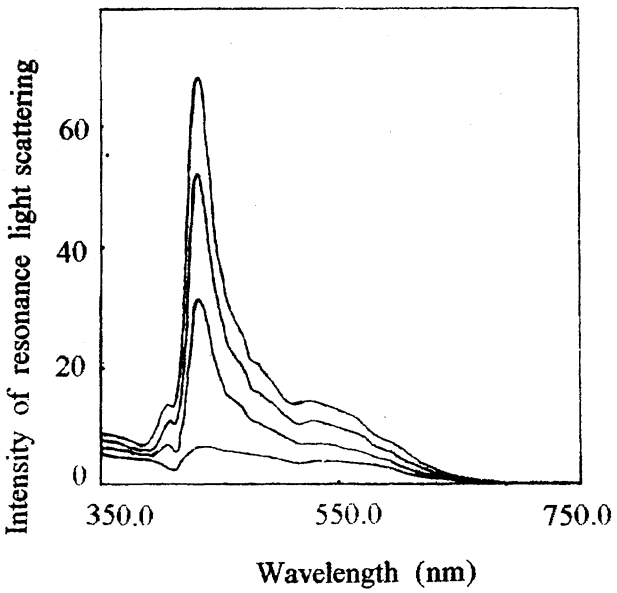

Fig. 6. Resonance light scattering spectrum as a function of the concentration of a nucleic acid. pH 7.48; Concentration: $[H_2tapp]^{4+}$, 1.2×10^{-6}; CTDNA, from top to bottom (10^{-7} M), 9.0, 6.6, 3.0, 0.0.

tion of the porphyrin is sufficiently large, which means that the porphyrin–porphyrin interaction occurs at a high concentration of the porphyrin. When $R > 0.89$, the intensity of the resonance light-scattering decreases, as shown in Fig. 5. It can also be recognized that the maximum wavelength of resonance light scattering is red-shifted, and that the exciton splitting signal becomes much more significant.

The enhancement effect of nucleic acids on the resonance light scattering of $[H_2tapp]^{4+}$ depends on the concentration and the kind of nucleic acids. Figure 6 shows an increasing enhancement of resonance light scattering with the concentration of nucleic acids. Table 1 lists the dependence of the resonance light-scattering intensity on the concentration and kind of nucleic acids. It also shows that the resonance light-scattering intensities increase linearly with the concentration of CTDNA and FSDNA in the range 1.8×10^{-7}—18.0×10^{-7} M, while with the concentration of YRNA in the range of 1.8×10^{-7}—18.0×10^{-7} M. In addition, there are some slight shifts of the resonance light-scattering peak along with variations of the concentration and kind of nucleic acids, particularly with the variations of the concentration of yeast RNA, as summarized in Table 2. It is worth noting that the wavelength-shift trends can be obtained repeatedly with increasing the concentration of nucleic acids, and can thus be disregarded that the data presented in Table 2 were experimental errors, even if a slit width of 5 nm was used. There might be some relationship between the maximal resonance light-scattering peak and the concentration of nucleic acids.

Formation of the Suprahelical Helixes of Nucleic Acids. As shown in Fig. 5, a maximal enhancement of resonance light scattering was obtained when R was 0.89. Fiel et al.[17] once proposed that the porphyrins with their charge of +4 could feasibly act as condensing agents, particularly at higher values of R and low ionic strength, and that a suprahelical helix of a nucleic acid could be formed. Similarly, Pasternack et al.[32] also supposed that a helical arrangement of the porphyrin molecules may occur on the nucleic acid polymer, which results in a helical alignment of the porphyrin transition dipole moments. When light interacts with this helical structure, a resonance phenomenon between the interacting porphyrin units occurs.[18] It is thus reasonable that the enhanced resonance light-scattering should be responsible for the formation of the suprahelical helixes of nucleic acids. Indeed, the exciton nature of the resonance light scattering of the bound $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ to nucleic acids suggests that some porphyrin–porphyrin interactions occur.[32] It is pos-

Table 1. Dependence of Resonance Light Scattering Intensity on the Concentration and the Kind of Nucleic Acids

Nucleic acids	Linear range ($\times10^{-7}$ M)	Linear regression equation ($c\times10^{7}$ M)	Correlation coefficient (r)
CTDNA	1.8—10.8	$I_{RLS} = -6.8+6.0c$	0.9962
FSDNA	1.8—10.8	$I_{RLS} = -6.8+5.9c$	0.9962
YRNA	1.8—18.0	$I_{RLS} = -8.7+3.9c$	0.9963

Concentration of $[H_2tapp]^{4+}$, 1.2×10^{-6} M; pH 7.48. c is the concentration of nucleic acids.

Table 2. Wavelength Shifts (nm) of Maximal Resonance Light Scattering with R

R	6.67	3.33	2.22	1.67	1.33	1.11	0.95	0.83	0.74	0.67
CTDNA	432.0	434.0	433.5	433.0	432.0	432.0	432.0	431.5	431.5	431.5
FSDNA	432.0	433.0	434.0	432.5	432.0	432.0	432.0	432.0	431.0	431.5
YRNA	432.0	434.0	436.0	438.0	438.0	438.0	438.0	436.5	436.5	436.0

Concentration of $[H_2tapp]^{4+}$, 1.2×10^{-6} M; pH 7.48.

sible that the porphyrin–porphyrin interactions, which may be indicative of a tendency for the aggregation of porphyrins, is promoted by nucleic acids in the way that $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ stacks along the surface of nucleic acids, namely the long-range assembly.[14] The assembly does not involve in the intercalation of $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ into the base pairs and binding with nucleic acids in the major or minor groove, but is mediated by the polyanion on the backbone of nucleic acids in accord with the arrangement of its negative charges. Thus, $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ appears to be indicating the suprahelical sense of the nucleic acid aggregate to which the porphyrin species is bound.

When R is lower or higher than 0.89, the maximal enhanced intensity of resonance light scattering decreases. It is possible that an increase in the concentration of nucleic acids decreases the mutual interactions of the titled porphyrin, and leads to a decrease in the formation probability of the suprahelical helixes. When R is low, $[H_2tapp]^{4+}$ only stacks along the surface of nucleic acids in the "face-on" and "edge-on" modes.[16,17] However, if the concentration of $[H_2tapp]^{4+}$ is too high, the molecular absorption resulting from $[H_2tapp]^{4+}$ becomes very strong, and leads to a reduction in the enhancement effect of nucleic acids. Thus, only at an appropriate concentration of $[H_2tapp]^{4+}$ or $[H_4tapp]^{6+}$ can the resonance light-scattering signals, including exciton splitting, resulting from the formation of suprahelical helix of nucleic acid, be sensitively observed.

Depending Factors on the Formation of the Suprahelical Helixes. The formation of the suprahelical helixes is affected by the pH and ionic strength of the aqueous solution, and the state of nucleic acids. Figure 7 shows that the formation of suprahelical helixes is scarcely affected if the pH value is higher than 4.1. When the pH is lower than 4.1 the suprahelical helixes still exist, as can be seen in Fig. 4, although two protons attach to each pyrrolic nitrogen atom in the porphyrine macrocycle, leading to a shift of the Soret band from 412.0 to 430.8 nm. However, differences in both the maximal resonance light-scattering peak and the exciton splitting signal are observed between the resonance light-scattering spectra of the suprahelical helixes induced by $[H_2tapp]^{4+}$ and by $[H_4tapp]^{6+}$. The maximal light-scattering appears at 452 nm and exciton splitting appears at 428 nm for the suprahelical helixes induced by $[H_4tapp]^{6+}$.

Although $[H_2tapp]^{4+}$ can bind nucleic acids even if the ionic strength is higher than 1.0 M ($K_{int}=4.2\times10^4$),[16] the suprahelical helixes can form only in low ionic-strength media. Figure 8 shows that the intensity of resonance light-scattering decreases rapidly with increasing the ionic strength. However, there is no remarkable change in the pattern of the

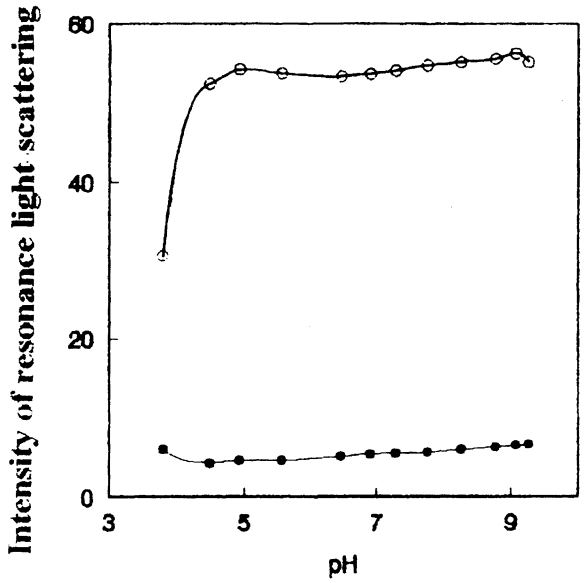

Fig. 7. Dependence of the formation of the suprahelical helix on pH. (○) DNA–$[H_2tapp]^{4+}$; (●) $[H_2tapp]^{4+}$. Concentration: CTDNA, 9.0×10^{-7} M; $[H_2tapp]^{4+}$, 1.2×10^{-6} M. Ionic strength, 0.004. All the data were obtained at 432.0 nm.

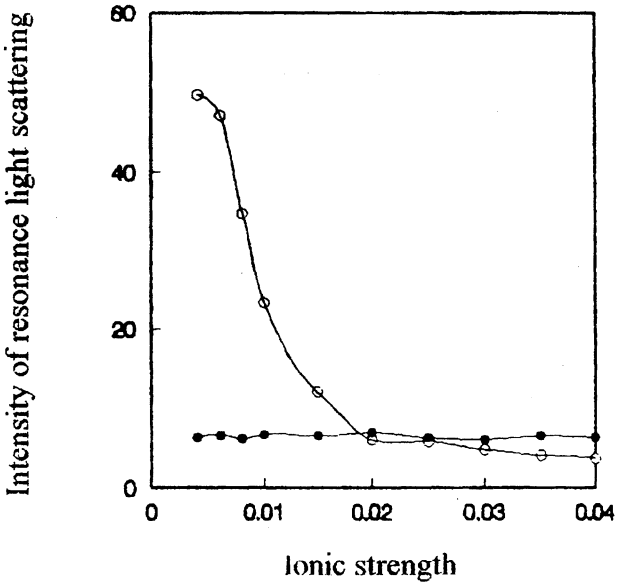

Fig. 8. Effect of ionic strength on the formation of the suprahelical helix. Symbols are the same as in Fig. 7. pH 7.48. All the data were obtained at 432.0 nm.

resonance light-scattering spectrum. The effect of the ionic strength may be due to the electrostatic shielding effect between the phosphate anions on the backbone of nucleic acids with the ionic-strength controller, which is unfavorable for the modulation of nucleic acids in the porphyrin interactions.

Although no variations of the resonance light-scattering spectrum pattern were observed for the suprahelical helixes of thermally denatured nucleic acids, the intensity of the resonance light-scattering was anomalous, and the reproducibility was not good. The reason may be ascribed to a conformational change of the nucleic acids. Since thermally denatured nucleic acids have anomalous thread mat structures, the suprahelical helixes can not be formed normally.

The initial concentrations of $[H_2tapp]^{4+}$ and nucleic acids have great effects on the formation of the suprahelical helixes. Different volumes of water were added into a 0.60 ml (1.5×10^{-5} M) aqueous solution of nucleic acids before adding $[H_2tapp]^{4+}$ and a buffer solution. As a results, the intensity of resonance light scattering changes with the added volume of water, as shown in Fig. 9. In this figure, all of the data are plotted by expressing the different concentrations of nucleic acids as a final concentration of 9.0×10^{-7} M. However, the fluorescence quenching of nucleic acids on $[H_2tapp]^{4+}$ is not affected by the initial dilution of nucleic acids,[25] which may be because the porphyrin–porphyrin interaction of the suprahelical helixes is not strong enough to quench the fluorescence concerning the initial concentration effect.

Conclusion

When the ionic strength is low, 5,10,15,20-tetrakis[4-(trimethyammonio)phenyl]porphyrine, both in its free base species, $[H_2tapp]^{4+}$ and its protonated species, $[H_4tapp]^{6+}$, can stack along the surface of nucleic acids. If the molar ratio of $[H_2tapp]^{4+}$ to nucleic acids is sufficiently large, suprahelical helixes can be formed. The formation of suprahelical helixes leads to an enhancement of the resonance light scattering with the maximal peaks being located on the longer wavelength side of the Soret-band region of the free-base or protonated porphyrin species. With resonance light-scattering spectroscopy, the suprahelical helixes can be monitored, and exciton splitting signals can be clearly observed. The exciton nature reveals that the formation mechanism of the suprahelical helixes is the porphyrin–porphyrin interaction on the surface of nucleic acids.

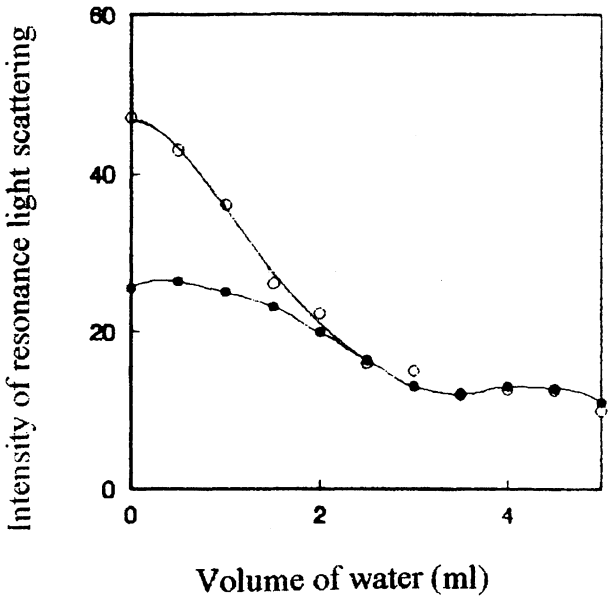

Fig. 9. Effect of water addition on the formation of the suprahelical helix. (○) CTDNA, (●) YRNA. Concentration: nucleic acids, 9.0×10^{-7} M; $[H_2tapp]^{4+}$, 1.2×10^{-6} M. All the data were obtained at 432.0 nm.

This program was support by the National Natural Science Foundation of the People's Republic of China (NSFC), and all authors here expressed their deep thanks.

References

1) R. J. Fiel, J. C. Howard, E. H. Mark, and N. Datta-Gupta, *Nucleic Acids Res.*, **6**, 3093 (1979).
2) R. D. Levere, Y. F. Gong, A. Kappas, D. J. Bucher, G. P. Wormser, and N. G. Abraham, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 1756 (1991).
3) L. Ding, G. Etemad-Moghadam, and B. Meunier, *Biochemistry*, **29**, 7868 (1990).
4) R. J. Fiel, T. A. Beerman, E. H. Mark, and N. Datta-Gupta, *Biochem. Biophys. Res. Commun.*, **107**, 1067 (1982).
5) G. D. Dougherty, *J. Inorg. Biochem.*, **34**, 95 (1988).
6) Y. Nonaka, D. S. Lu, A. Dwivadi, D. P. Strommen, and K. Nakamoto, *Biopolymers*, **26**, 999 (1990).
7) Y. X. Liu, A. Koningstein, and Y. M. Evdokimov, *Can. J. Chem.*, **69**, 1791 (1991).
8) B. P. Hudson, J. Soe, D. J. Berger, and D. R. McMillin, *J. Am. Chem. Soc*, **114**, 8997 (1992).
9) R. F. pasternack, E. J. Gibbs, and J. Villafranca, *Biochemistry*, **22**, 2406 (1983).
10) R. F. pasternack, E. J. Gibbs, and J. Villafranca, *Biochemistry*, **22**, 5409 (1983).
11) E. J. Gibbs, M. C. Maurer, J. H. Zhang, W. M. Reiff, D. T. Hill, M. Malicoka-Blaszkiewiccz, R. E. McKinnie, H. Q. Liu, and R. F. Pasternack, *J. Inorg. Biochem.*, **32**, 39 (1988).
12) M. F. Lin, M. H. Lee, K. T. Yue, and T. C. Mazilli, *Biochemistry*, **32**, 3217 (1993).
13) B. Mueunier, *Chem. Rev.*, **92**, 1411 (1992).
14) R. F. Pasternack, C. Bustamante, P. J. Collings, A. Giannetto, and E. J. Gibbs, *J. Am. Chem. Soc.*, **115**, 5393 (1993).
15) M. A. Sari, J. P. Battioni, D. Dupre, D. Mansuy, and J. B. Le Pecq, *Biochemistry*, **29**, 4205 (1990).
16) M. J. Carvlin, N. Datta-Gupta, and R. J. Fiel, *Biochem. Biophys. Res. Commun.*, **108**, 66 (1982).
17) M. J. Carvlin and R. J. Fiel, *Nucleic Acids Res.*, **11**, 1621 (1983).
18) D. L. Akins, *J. Phys. Chem.*, **90**, 1530 (1986).
19) D. L. Akins, C. K. Akpabli, and X. Liu, *J. Phys. Chem.*, **93**, 1977 (1989).
20) R. F. Pasternack and P. J. Collings, *Science*, **269**, 935 (1995).
21) G. A. Miller, *J. Phys. Chem.*, **82**, 616 (1978).
22) J. Anglister and I. Z. Steinberg, *Chem. Phys. Lett.*, **65**, 50 (1979).

23) S. G. Stanton and P. Pecora, *J. Phys. Chem.*, **75**, 5615 (1981).
24) J. C. Paula, J. H. Robblee, and R. F. Pasternack, *Biophys. J.*, **68**, 335 (1995).
25) R. F. Pasternack and K. F. Schaefer, *Inorg. Chem.*, **33**, 2062 (1994).
26) C. Z. Huang, K. A. Li, and S. Y. Tong, *Anal. Chem.*, **68**, 2259 (1996).
27) C. Z. Huang, K. A. Li, and S. Y. Tong, *Anal. Chem.*, **69**, 514 (1997).
28) C. Z. Huang, Ph. D. Dissertation, Peking University, Beijing (1996).
29) Z. Chem, J. Liu, and D. Luo, "Biochemistry Experiments," Chinese University of Science and Technology Press, Hefei (1994).
30) Y. E. Zeng, Y. E. Zhang, and Z. H. Chen, "Handbook of Modern Chemical Reagents," Chemical Industry Press, Beijing (1989), Vol. 4.
31) M. Gouterman, in "The Porphyrins," ed by D. Dolphin, Academic Press, New York (1978), Vol. 3.
32) E. J. Gibbs, I. Tinnoco, Jr., M. F. Maestre, P. A. Ellinas, and R. F. Pasternack., *Biochem. Biophys. Res. Commun.*, **157**, 350 (1988).