Studies on the Binding Mode of Pinacyanol Chloride to Nucleic Acids

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The interaction of pinacyanol chloride (PC) with nucleic acids has been investigated by a series of experiments. Extensive hypochromism, appreciable peak shifts, isosbestic points and new peaks of the product of binding to nucleic acids in the spectra were observed. They showed that the interaction between PC and nucleic acids occurred. The results from absorption spectra of DNA, DNA melting, electrophoresis and fluorescence polarization studies have indicated that PC binds to DNA in nonintercalative way. Consistent with the nonintercalation, the studies of fluorescence titration and absorption titration specified that the binding of PC to nucleic acids occurred by an outside stacking binding, in which nucleic acids served for acting templates. The fact that the new absorption peaks of bound PC at *ca.* 485 nm are just close to the absorption bands of H-aggregate of PC at high concentrations without DNA further supports the outside stacking binding mode. In addition, other evidence indicated that the interaction between PC and nucleic acids is not purely electrostatic.

Keywords pinacyanol chloride, nucleic acids, binding mode

The fundamental work of Peacocke and Skerrett[1] suggested that the binding of dyes to nucleic acids occurs by two principal types termed as type I and type II.[2] Type I corresponds to the monomer binding which occurs in that the dye is placed in one of the grooves of DNA (type I_1) or between adjacent base pairs (type I_2, intercalation). It depends on the dye structure whether the binding takes place as double-strand or single-strand[3] intercalation. While type II corresponds to an outside stacking binding without base specificity and can form longer polymer units.

Pinacyanol chloride (PC) is a singly charged cyanine dye (Fig. 1). Its combination mode with nucleic acids is of interest because of the mutagenicity in yeast. Rare studies on the interaction of PC with DNA have been reported. Lerman[4] supposed that PC interacts with DNA in nonintercalative way, only based on yielding lowered viscosity and increased sedimentation coefficient. In contrast to Lerman,[4] Kodama *et al.*[5] determined only the orientation of a bound species relative to the helix axis by utilizing flow dischroism technique. However, two important aspects have been neglected in these studies. First, one kind of data alone was clearly incapable to exactly resolving the binding modes for the complex as established by several different lines of evidence.[6] Second, what kind of nonintercalation does exist remain to be clarified.

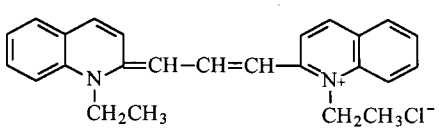

Fig. 1 Structure of pinacyanol chloride.

In this paper, a series of experiments designed to investigate the binding mode between PC and nucleic acids in more detail were reported. The results have

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shown that PC-nucleic acids interaction is through type II, an outside stacking binding without base specificity in which nucleic acids serve for acting templates.

Experimental

Materials

Calf thymus DNA (ctDNA), yeast RNA (yRNA) and PBR 322 DNA/EcoR I were obtained from Sino-American Biotechnology Company. ctDNA and yRNA were directly dissolved in water and stored at 4 ℃. Their concentrations were determined spectrophotometrically[7] using the following extinction coefficients at 260 nm: ct DNA, 0.020 $mL \cdot \mu g^{-1} \cdot cm^{-1}$; yRNA, 0.024 $mL \cdot \mu g^{-1} \cdot cm^{-1}$. λDNA/Hind III was purchased from MBI Fermentas Company. Pinacyanol chloride and ethidium bromide (EB) were obtained from Aldrich Chemical Company and Sino-American Biotechnology Company, respectively. Organic polyanions, sodium polyacrylate and sodium polymethylacrylate, were kindly offered by Polymer Chemistry Laboratory of Nankai University. The thermally denatured ctDNA was prepared by heating the native ctDNA in boiled water bath for 30 min and immediately cooled in ice/water bath.

Tris-HCl buffer solution (pH 7.3) and TAE buffer solution which contained 0.04 mol/L tris-acetic acid and 0.001 mol/L EDTA were used.

To diminish adsorption of PC to the glass walls of volumetric apparatus and that of the measuring cells, a small amount of ethanol (a final concentration of 6.0% *V/V*) was added into PC aqueous solution.

All other chemicals were of analytical reagent grade or better and deionized distilled water was used throughout.

Procedure

The absorption spectra recordings and absorption measurements were conducted on a Shimadzu UV-240 ultraviolet-visible spectrophotometer. The fluorescence spectra and fluorescence intensities were taken from a Shimadzu RF-540 spectrofluorometer. Absorption titration as well as fluorescence titration with nucleic acids was performed, while keeping the concentration of the dye PC constant, and by varying the polyanions concentrations. The fluorescence polarization measurements were made on a Shimadzu RF-540 spectrofluorometer with a pair of polarizers. The DNA melting experiments were carried out on a Shimadzu UV-365 UV-VIS-NIR spectrophotometer with a temperature controller SPR-8.

Electrophoresis was run on a DF-C horizontal slab gel electrophoresis apparatus (Dongfang Teli Kemao Center, Beijing, China). The working voltage was set at 15 V and the lengths of the slab and the gel were 17 cm and 10 cm, respectively. The running gel for electrophoresis was 0.8% agarose. Electropherograms were acquired from an 80-6247-01 Imagemaster VDS (Phamaciabiotech, Swiss).

Results and discussion

Absorption spectral studies

The electronic absorption spectra of PC in the presence of ctDNA were illustrated in Fig. 2a. Strong decreases in the intensities of the 598 nm and 548 nm peaks were found when various amounts of ctDNA were added to the PC solution. The absorption spectra clearly showed that the red and blue shifts. The absorption maxima were shifted from 598 nm and 548 nm for the free PC to 594 nm and 560 nm for the bound PC in the presence of ctDNA, respectively. Accompanying the binding process, the new absorption peak at *ca*. 485 nm appeared. Over a range of concentrations, two isosbestic points appeared in the family of absorption curves, one at 506 nm and the other at 624 nm. Over this range of concentrations, according to the well-known properties of the isosbestic point, two colored species are in equilibrium. The addition of further amounts of the DNA to the dye solution did not cause any additional changes in the absorption spectra, owing to the saturation of binding of the dye to ctDNA. Similar phenomena were also exhibited in the absorption spectra of PC-yRNA system.

Alternatively, the absorption of the base pairs of ctDNA at 260 nm revealed obvious hyperchromicity as the concentration of PC increased (see Fig. 2b). Such an increase in absorption argues against the intercalative binding can give rise to hypochromicity as proposed[8,9] since intercalation in the DNA helix would strengthen the stability of DNA helix. From the above observations, we surmised that nonintercalation occurred upon binding of PC to DNA.

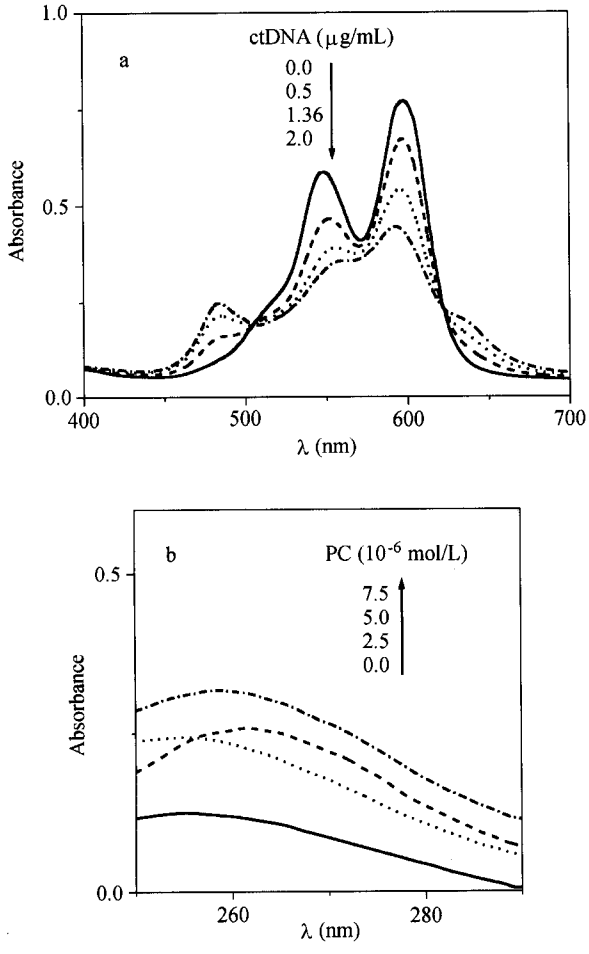

Fig. 2 Absorption spectra of (a) PC in the presence of different amounts of ctDNA and (b) ctDNA in the presence of different amounts of PC. (a) PC, 7.0×10^{-6} mol/L; (b) ctDNA, 0.85 μg/mL; pH = 7.3; cell, 1.0 cm.

DNA *melting studies*

In order to test further whether or not the nonintercalation exists between PC and DNA, ctDNA melting studies were monitored by following the UV absorbance of the DNA at 260 nm as a function of temperature with or without PC. Owing to the increased stability of the helix in the presence of an intercalator, intercalation into the helix denatures by as much as 5—8 ℃.[10,11] Such large increases in the melting temperature were not observed if the dye binds in nonintercalation.[12] Our experiment data in ctDNA melting studies revealed that, at 260 nm, the DNA absorbance as a function of the temperature displayed a clear transition at 75 ℃ with or without PC. This supports the proposition of nonintercalation mentioned above.

Electrophoresis studies

Additional evidence in support of the nonintercalation mechanism was provided by comparing three sets of PBR 322DNAs/EcoR I, *i. e.*, DNA, PC + DNA and EB + DNA, which were run using the same gel with the same field strength. Electropherograms was shown in Fig. 3. For EB, a known well DNA intercalator, the mobility of EB-DNA may result from three factors: (1) the greater rigidity because of intercalation,[13] (2) the less negative charge that the EB-DNA complex possessed and (3) the higher molecular weight of EB-DNA complex. As the binding affinity of dye with DNA in a nonintercalative way was too weak to counteract well the electrical field force during electrophoresis, the apparent mobility of dye-DNA in a nonintercalative way is almost the same as that of free DNA.[9] From Fig. 3, it can be seen that the mobility order is as follow: DNA ≈ PC-DNA ≫ EB-DNA, *i. e.*, the difference between PC-DNA and DNA is much smaller than that between EB-DNA and DNA. We consider the minor mobility difference observed between PC-DNA and DNA originates from nonintercalation of the dye.

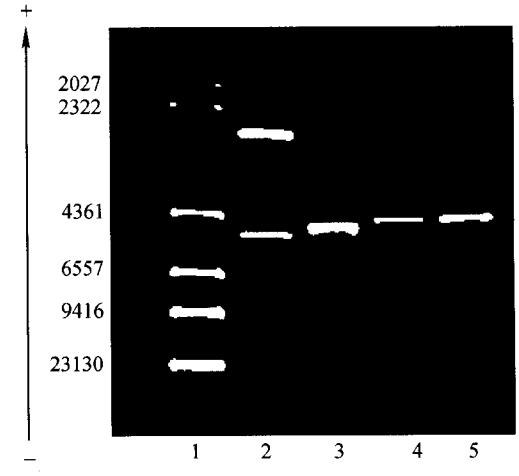

Fig. 3 Electropherogram. Lane 1: Marker λDNA/Hind III; Lane 2: PBR 322 DNA; Lane 3: PBR 322 DNA/EcoR I + EB; Lane 4: PBR 322 DNA/EcoR I + PC; Lane 5: PBR 322 DNA/EcoR I; PBR 322 DNA, 0.5 μg; PC, 0.6 μg; EB, 0.6 μg.

Fluorescence polarization studies

We also followed fluorescence polarization experi-

ment by a Shimadzu RF-540 spectrofluorometer. Fig. 4 showed the effect of increasing PC concentration on the polarization of EB bound to DNA. It should be noted that an intercalatively bound form of EB, held more rigidly in the helix with long residence times on the time scale of the emission lifetimes, should yield finite polarization, while free form of EB or a more flexible surface binding along the groove would not contribute significantly to the polarization in the test media.[14,15] Given that, like EB, PC intercalates into the helix of DNA, it would compete against EB for over the intercalation sites in DNA, and thus leads to a decrease in polarization of EB-DNA complex system. In fact, as expected for a non-displacement effect, the polarization of EB-DNA complex system was found to keep constant first and then increase with the increasing of PC content over this range of concentrations. Clearly, the constant or increasing in polarization further supported the nonintercalative binding.

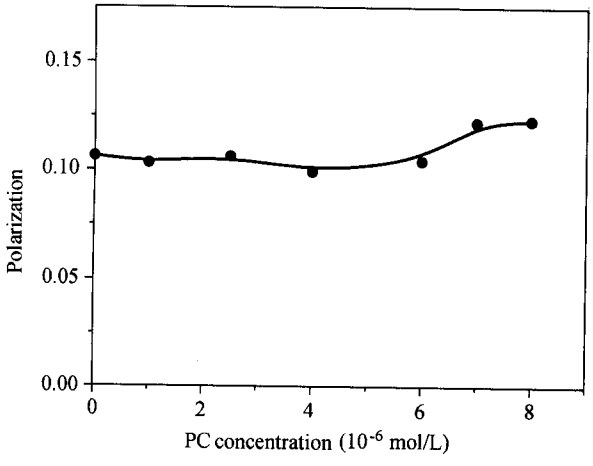

Fig. 4 Competitive binding between PC and EB for ctDNA. ctDNA: 0.76 μg/mL; EB: 5 μg/mL; pH = 7.3; λ_{ex} = 540 nm; λ_{em} = 590 nm.

Studies of fluorescence titration

Until now, our studies as well as the results reported by Lerman[4] have proved that the nonintercalation does exist between PC and DNA, but not specified what kind of nonintercalation yet. Under different conditions, the fluorescence intensities of PC system were measured. A result with little or no enhancement of emission was observed as the increasing of ctDNA. It should be noted that, the enhancement of fluorescence of cyanine dye solution in the presence of DNA is supposed to be caused by a restriction of rotation around the methine bond between heterocycles in a cyanine dye molecule.[16,17] Moreover, groove binding or intercalation of dye to the helix can inhibit the rapid rotation around various bonds.[15,18] As a result, further conclusion was inferred that PC probably prefers binding to DNA in type II over type I since type II less restricts the rotation.

Studies of absorption titration

Thermal denaturation of the DNA disrupts the ordered arrays of base pair in the double helix and unwinds its double helix structure, apart from limited local renaturation. Without regard to the possibility of intercalation, if PC binds to DNA in the grooves, thermal denaturation of the DNA would be expected to remove or, at least, greatly to reduce such binding. Our observations (see Fig. 5) showed that this was not the case and that the binding process was markedly increased in extent by denaturation, *i.e.*, PC binding with nucleic acids is not specific for double-stranded and it binds more readily to denatured ctDNA when compared with natured ctDNA. It is natural to suppose that, being different from the type I interaction modes of grooving and intercalation binding, PC binds by outside stacking binding without base specificity (type II) and form longer polymeric units, in which nucleic acids serve for acting templates.[19,20]

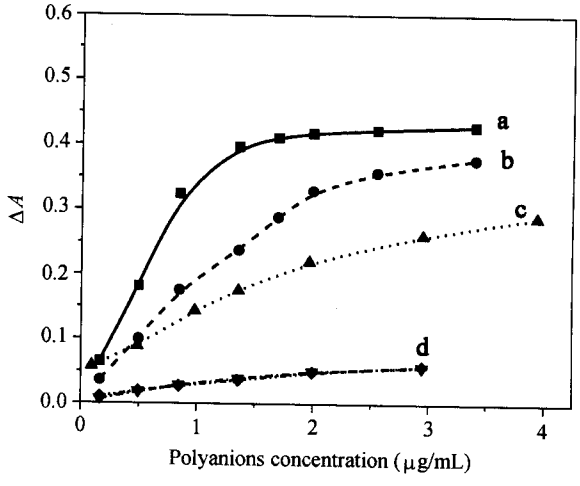

Fig. 5 Titration curves for (a) thermally denatured ctDNA, (b) ctDNA, (c) yRNA and (d) sodium polyacrylate or sodium polymethylacrylate. PC: 7.0×10^{-6} mol/L; pH = 7.3; λ = 598 nm; cell, 1.0 cm. The decreased absorbance was defined as $\Delta A = A_0 - A$, where A_0 and A were the absorbances of PC without and with polyanions, respectively.

In Fig. 6, absorption spectra of PC solutions without ctDNA were shown. As the dye concentration increased, both the intensity ratios of the H-aggregate band and dimer band the H-aggregate band and monomer band increased,[21] which accompanied ablue shift over 5—6 nm. On the other hand, passing on to the PC solution with DNA, an obvious increase in the intensity of the new absorption peaks of bound PC at *ca*. 485 nm and a decrease in the intensities of dimer band and monomer band were also observed (see Fig. 2a). It is interesting to note the fact that the new absorption peaks of bound PC with DNA are just close to the absorption bands of greater aggregation (H-aggregate) of PC without DNA, and are blue-shifted compared to the latter (Fig. 6). However, the more the position of the H-aggregate absorption band is blue-shifted, the greater the number of species contained in the aggregate.[22] Thus, the presence of DNA favors the creation of the H-aggregate with a greater number of species, and /or H-aggregate created on the DNA molecule has a different structure and, therefore, a different energy level. However, in any case H-aggregates of dyes are formed on the DNA molecule. This further proves our earlier statement that PC binds to nucleic acids by type II.

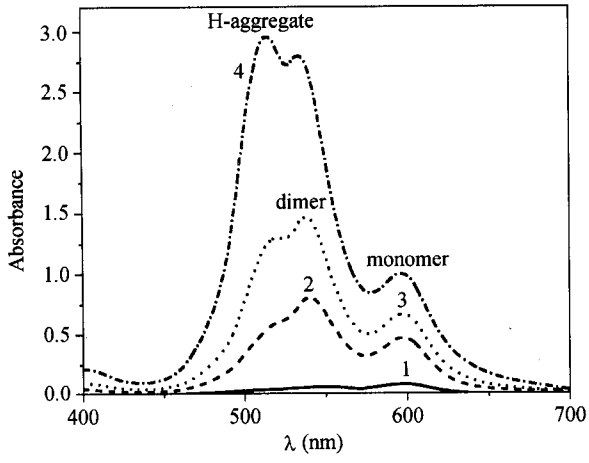

Fig. 6 Absorption spectra of PC solutions in the absence of DNA. Concentrations of PC: (1) 10^{-5} mol/L; (2) 10^{-4} mol/L; (3) 2×10^{-4} mol/L; (4) 4×10^{-4} mol/L. pH = 7.3; cell, 0.1 cm.

Fig. 5 also revealed that the interaction between PC and nucleic acids is not supposed to be purely electrostatic. This comes from PC binding to organic polyanions such as sodium polyacrylate or sodium polymethylate with the evidence of minor absorbance decrease, in this case binding due only to electrostatic interaction.

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