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Spectroscopic Study of the Interaction between New Fluorescent Dyes with Nucleic Acids

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Abstract: A series of new dansylamide derivatives have been synthesized and the specific binding affinity of such fluorophores to nucleic acids has been investigated by using absorption, circular dichroism (CD), fluorescence and atomic force microscopy (AFM). The results indicate that the positive charge of the ligand and the π-π stacking between the dansyl part of the ligand and the DNA base pair may play an important role when binding to polynucleotides.

Keywords: Spectroscopic study, fluorophors, complexation, nucleic acids.

Considerable effort has been continuing to focus on the development of new fluorescent dyes to recognize nucleic acids[1-3]. Although dansyl is a well-known sensitive hydrophobic probe which has been widely utilized as a fluorescent probe for the study of proteins, yet little effort has been focused on the exploring dansylamide derivatives which may have specific effects on nucleic acids. Since the binding affinity of such fluorophores to polynucleotides was greatly affected by their sidechain substitutions, in this work several new dansyl derivatives with specific binding to nucleic acids have been studied.

In the experiments, biochemical materials were purchased from Aldrich and Pharmacia. Fluorescence and UV-Vis absorption spectra were obtained with a Hitachi Fluorescence Spectrophotometer F-2000 and a Uvikon 860 from Kontron, respectively. A Nanoscope III (Digital Instruments) was utilized for AFM measurements which were performed in the tapping mode in air at 20°C. CD spectra were obtained with a JASCO J-715 Spectrophotometer interfaced to a PC. All curves are the average of three scans.

Ligands such as Bis-(Dansyl-N,N´-3-aminopropyl)-piperazin (1), Methylated Bis-(Dansyl-N,N´-3-aminopropyl)-piperazin (2), Dansyl-3-dimethyl aminopropylamine (3), Dansyl 3-trimethylamino propylamine (4), Tris (1-dansyl ethyl-2) amine (5), Bis Dansyl-diamino-p-xylene (6), Bis Dansyl-phenylendiamine (7) are a series of new ligands synthesized as previously reported[3], which possess one, two or three dansyl units connected by polyamines with and/or without positive charges. The emission and excitation wavelengths of these new fluorescent dyes indicate some difference from each other due to the different polyamine spacer that is connected to the dansyl chromophore.

The spectroscopic studies of the host ligands complexed with polynucleotids illustrate different behaviour from ligand to ligand. It is observed that upon addition of CT-DNA and/or denatured, single-stranded CT-DNA, the fluorescence intensities of

ligands 5, 6 and 7 were found to decrease significantly and could reach a final saturation value with the increasing of DNA concentration from 10^{-8} to 10^{-5} M, accompanied with no shifts of the fluorescence peaks; while ligands with only one dansyl unit, *i.e.*, ligand 3 and 4, show relatively low affinity with a little increase of fluorescence intensity, and there is also no any shift of the peak wavelength. In contrast, the fluorescence of ligand 1 decreases considerably with the blue shift (*ca.* 20 nm) of emission wavelength upon the increase of the DNA concentration; while for ligand 2, binding with biopolymers is found to involve two steps, *i.e.*, the first step observed for the decrease of fluorescence intensity without peak shift at base pair (bp) concentration of $0.0<[bp]<10^{-7}$M with a low ratio of r =[bp]/[2](r≤0.01), and the second phase at higher ratio of [bp]/[2], accompanied with significant increase of fluorescence intensity and blue shift of the emission wavelength (*ca.* 29nm).

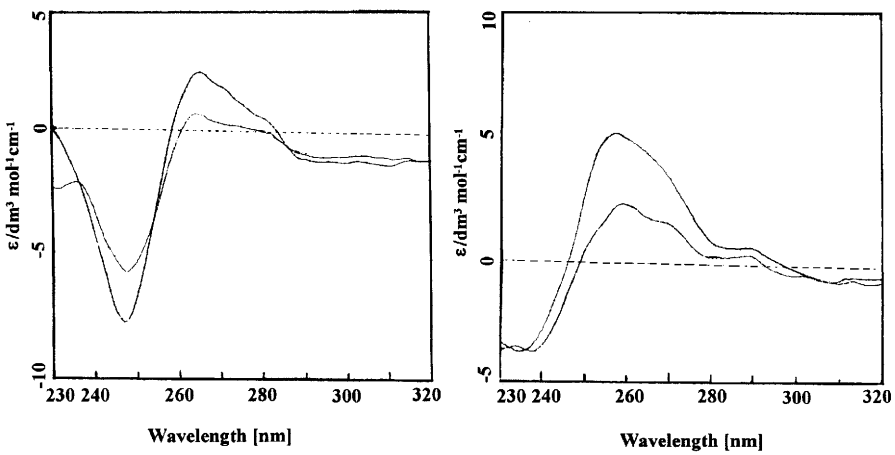
An additional evidence of binding mode to biopolymers comes from absorption spectroscopic study. It is found that for ligand 2, addition of biopolymers like CT-DNA produced strong decreases of absorption, accompanied by a large red shift of the peak to a position which is similar to that of ligand 2 in organic solvents like methanol or ethanol. In addition, an isosbestic point is observed at 299 nm, which is shifted to 307 nm at higher concentration of CT-DNA. In comparison, the absorption of compound 1 also decreases considerably with the increasing of polymer concentration, but almost no peak shift occurs in this case. For compound **3** and **4**, a relatively small decrease of absorption is also observed upon binding to DNA. However, the solubility problems of the complex with compound **5**, **6** and **7** prevent the observation of corresponding absorbance changes.

Other DNA binding evidence is illustrated in circular dichroism (CD) spectroscopy which is conducted at 20°C. It is observed that the CD spectra for biopolymers exhibit an apparent decrease of the peak intensity upon addition of the host ligands; meanwhile, the CD spectroscopical changes in the UV range induced by ligand 2 show larger decrease of intensity than those with other dansyl derivatives. Besides, the binding also indicates specific affinity to single-stranded PolyA for the ligands with two or three dansyl units. **Figure 1** is the CD spectra of some polynucleotides including Poly (dA-dT) and Poly(dG). (dC) in the absence and presence of ligand 2. The significant decrease of the peak intensity upon complexation may be attributed to the interaction of the ligand with the asymmetric environment of the biopolymer.

Further evidence from thermal melting studies provides additional information pertaining to the binding modes of the ligands with biopolymers. The results indicate that with CT-DNA , at a ratio of 0.1 mol of ligand per mol of nucleic acid phosphate, the melting temperature increased by 2.3°C for ligand 2 and only increased 0.6°C for ligand 1; while for other dansyl derivatives, there are almost no change of the DNA melting temperature at the same ratio of the ligand to nucleic acid phosphate concentration. Further information at higher ratio of the ligand to DNA concentration could not be obtained due to the solubility problem of the complex. Nevertheless, in view of the studies above, it appears that in comparison with other dansyl derivatives, ligand 2 has different affinities and binding modes with biopolymers. And the fluorescence and

absorption studies clearly indicate that binding of ligand 2 with polymers is biphasic and proceed in two steps: the first step with large affinities at low ratio of [bp]/[2] ($\leq$0.01),

Figure 1. CD spectra of (a) 48 μ M Poly(dA-dT) in the absence (top) and the presence (bottom) of 12 μ M ligand 2; (b) 38 μ M Poly(dG)·Poly(dC) in the absence (top) and the presence (bottom) of 12 μ M ligand 2.

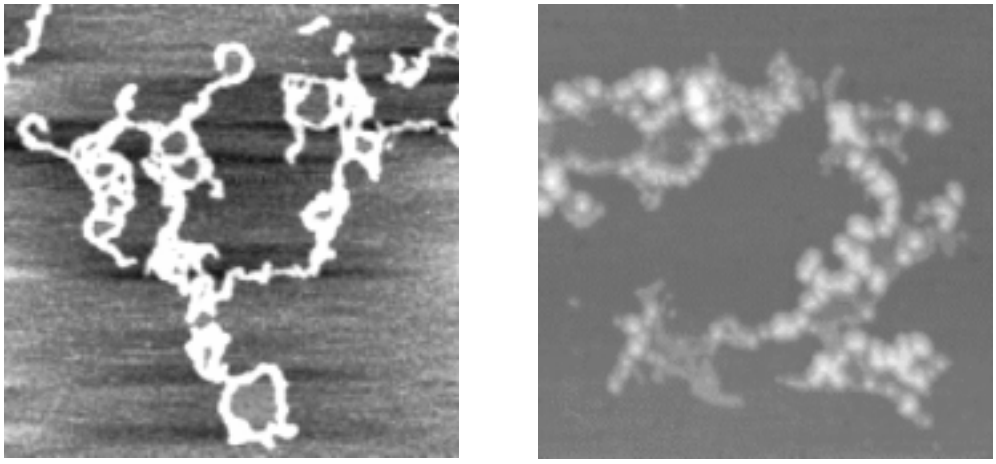


which may involve self-stacking of the dye induced by association in the major groove of double-strands,and the second step at higher polymer concentrations where fluorescence increases, which may exhibit at least partial intercalation of the dye into the helix and/or the specific binding of the dye to certain sites on the polymer; while ligands like 1, 5, 6 and 7 also exhibit large DNA affinities and may primarily involve groove binding. The apparent affinities with CT-DNA observed from fluorescence titrations indicate that for the groove binding of ligands 1, 5, 6, and 7, association lgK>7.0, while for weak binding with ligands 3 and 4, lgK<3.0. Especially, a competitive ethidium bromide displacement method was utilized to compare the overall DNA binding affinities. For instance, it is found that for ligand 1 and 2 with CT-DNA, the affinity values of C_{50}, which is based on fluorescence competiton measurements with ethidium bromide and conducted in the same conditions as the literature[4], are 1.0×10^{-5} and 5.0×10^{-6} M, respectively, indicating strong binding between these new ligands and nucleic acids.

Moreover, to further explore the mechanism of the ligand-DNA interaction, atomic force microscopy (AFM) has also been utilized to reveal the nanostructural information of the complex when binding to DNA. **Figure 2** is a typical topographical image of the complex of N-dansyl-3-(trimethylamino)propylamine (ligand 4) with CT-DNA at a ratio of r = 10 (r = [ligand]/[DNA]), which indicates that the packing particles have deposited in some fraction of the DNA chain. Although our previous work suggests that the binding of such a ligand to DNA is rather weak, yet AFM investigation clearly indicates that specific interaction also occurs upon complexation. The regular particles located on the helix chains may indicate that the π-π stacking between the dansyl part of the ligand and

the DNA base pair may make it easier for the interaction between helical segments to form the aggregated or condensed DNA structures.

Figure 2. Topographic view of AFM images of (a) CT-DNA (scan size 1500 nm × 1500 nm) (10μM), and (b) the complexes (scan size 1500 nm × 1500 nm) with compound 4 (r=10).



(a) **(b)**

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