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Evidence of DNA-Ligand Binding with Different Modes Studied by Spectroscopy

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Abstract: The binding behavior of several fluorescence dyes to calf thymus DNA has been studied by absorption, fluorescence and atomic force microscopy (AFM), which could provide direct evidence of formation modes and the corresponding nanostructural features of the ligand-DNA complexes.

Keywords: atomic force microscopy (AFM), absorption spectroscopy, fluorescence, DNA interaction

The study of DNA (deoxyribonucleic acid) conformational changes upon ligand binding is a hot spot in the frontier field of relative research in chemical, biological or medicinal areas.[1-5] The structural changes of DNA in response to different bound ligands have attracted continuing attention in the medicinal design of anticancer and/or anti-AIDS drugs. To further reveal the relationship between the specially functional structure of ligands and the conformational changes of DNA, the binding modes of different ligands with special structure to DNA have been explored in this study by primarily using absorption, fluorescence spectroscopy and atomic force microscopy (AFM).

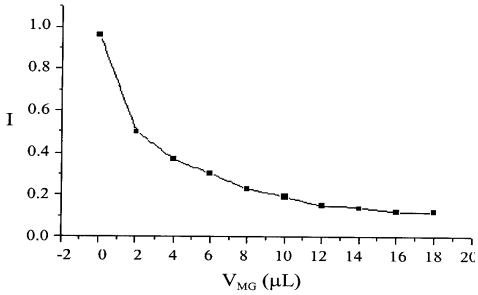
Calf thymus DNA (CT-DNA) was purchased from Sigma Chemical Co. and used without further purification. DNA stock solution was stirred or sonicated to shorten the length of CT-DNA. Other chemicals used were of analytical grade. Solutions were prepared with double-distilled water and the experiments were carried out as previously reported. 2

First of all, the different DNA binding modes with ligands including methylene green, fuchsin basic and rhodamine B, have been investigated by absorption spectrometric study. It is found that for rhodamine B, the increase of CT-DNA concentration produced strong decrease of absorption but without any shift of the peak position, indicating that groove binding occurs between rhodamine B and DNA. In contrast, the absorption of fuchsin basic increases considerably with the increase of biopolymer concentration, accompanied by a remarkable red shift of the peak (up to *ca.* 8nm) when the ratio of [bp]/[ligand]$\geqslant$10; while for methylene green, with the increase of DNA concentration, the absorption decreases significantly, accompanied by a larger red shift (up to *ca.* 16nm) of the peak when the ratio of [bp]/[ligand]$\geqslant$1. Therefore, the absorption spectroscopic changes of fuchsin basic and methylene green in the presence of DNA indicate that at lower ratio of [bp]/[ligand], the self-stacking of the ligand on the surface of double helix of DNA occurs during molecular interactions; while at higher ratio of [bp]/[ligand] as described above, the considerable red shift of the absorption peak suggests that the interactions involve at least partial intercalation of the ligand into the helix and the DNA binding with methylene green appears much stronger than that with fuchsin basic.

In comparison, the results of our fluorescent study provide the identical information for the DNA molecular interaction. **Figure 1** is a representative titration curve for the binding affinity (C_{50}) to DNA, which is based on the fluorescence competition

measurements with ethidium bromide (EB) and conducted in ways similar to previous studies in the literature[4]. The affinity values of C_{50} obtained for fuchsin basic and methylene green are $3.1X10^{-4}$ and $6.0X10^{-6}$ mol/L, respectively, suggesting the remarkably stronger binding of methylene green to DNA than that of fuchsin basic.

Figure 1 A competition titration curve for the binding affinity of methylene green to DNA. $[DNA]_0=2.0\times10^{-4}$mol/L; $[EB]_0=1.26\times10^{-6}$mol/L. The concentration of methylene green (MG) stock solution in tris buffer (10 mmol/L, pH 7.5) is 1.0×10^{-2}mol/L.



Further binding evidence comes from the nanostructural study by atomic force microscopy (AFM). It was observed in our study that for the intercalation ligands like methylene green and so on, the typical topographical image of the complex with DNA at a 1:1 concentration ratio ([DNA]=10^{-5} mol/L) involves the packing particles deposited in some fraction of the DNA chain; when the time exceeded about 1h after initiation of the above experiment or at a bigger ligand concentration, there were no DNA chains left and only big compact particles appeared in the image, suggesting that aggregation and/or condensation occurs in this phase. On the other hand, for the groove binding ligands like rhodamine B, the apparent packing particles could not be found on the DNA chain after complexation though the binding to the helix induced the big increase of dimension of the DNA chain; however, when the time exceeded about 2h after initiation of the experiment or at a much higher concentration ratio of ligand to DNA, then the aggregation or condensation process was observed and finally big particles with different shapes were left in the image. This phenomenon may indicate that groove binding ligands could also readily lead to the interaction between helical segments and make the biopolymers easily aggregate. In comparison, the intercalation ligands binding to DNA would induce significant structural variations of the DNA helix, which might favor the DNA packaging to finally cause the aggregation or condensation. Therefore, in view of our studies above, it appears that AFM study of the ligand-DNA interactions can not only reveal the nanostructural information of the complex but also facilitate the direct elucidation of the binding modes.

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