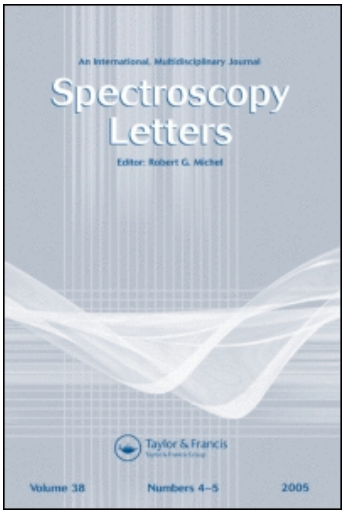

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STUDY OF INTERACTION OF BERBERINE WITH DNA IN THE PRESENCE OF β -CYCLODEXTRIN

KEY WORDS: DNA, β -CD, berberine, absorption spectra, fluorescence spectra

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ABSTRACT

In this paper, β -Cyclodextrin (β -CD) was used to study the interaction of berberine with DNA by the means of absorption spectrum and fluorescence spectrum. Experimental results showed that it was the isoquinolinic part that intercalated into the DNA double helix. And the value of the intrinsic binding constant K was 4.5×10^{3}l/mol in the presence of β -CD and 1.1×10^{4}l/mol in the absence of β -CD.

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Binding studies of small molecules with deoxyribonucleic acid(DNA), as are necessary for the design and synthesis of new and more efficient drugs target to DNA, are areas of great importance and interest.[1,2] Metal complexes, porphyrins, natural antibiotics and a host of other planar hetercyclic cations have been investigated for their DNA binding affinity. By far, most works have been focusing on small molecules' recognizing base pairs, sequences and /or conformational features of DNA.[3,4] The three models of binding of small molecules to DNA, intercalative binding, groove binding and electrostatic binding, have been widely accepted concepts. But little attention was paid to the small molecules themselves. In fact, it is also important to determine which parts of these small molecules interact with DNA for the rational design of drugs.

Cyclodextrins, a kind of polysaccharides made up of six to eight D-glucose monomers connected at the 1 and 4 carbon atoms, have been of great interests for their special structures and characters.[5,6] Many small molecules can be included into their cavities, forming inclusion complexes, which can be used in chemical analysis. Generally, cyclodextrins only include part of small molecules and the hydrophobic character of their cavities may cause the change of

absorption and/or fluorescence spectra of small molecules. So cyclodextrins are ideal molecules for the study of small ligand binding, especially for the understanding of binding of probe molecules to DNA and proteins.

So far, there are reports with regard to the interaction of berberine with DNA. Berberine is a good intercalator of DNA and it shows a high specificity to AT-rich DNAs.[7~9] But there is not enough information to tell which part of berberine intercalates into the DNA double hilex. Our experimental results indicate that β-CD can include the benzenoid part of berberine to form an inclusion complex, as is shown in FIG.1. At the same time the complex can interact with DNA through the isoquinolinic part of berberine.

EXPERIMENT SECTION

The absorption spectra measurements were made with a Perkin-Elmer Lamda-7 spectrophotometer while the fluorescence experiments were recorded on a Perkin-Elmer LS-50B spectrometer. In fluorescence measurements, all the solutions were excited at 348nm.

Berberine chloride was extracted from the rhizome of Chinese goldthread and was recrystallized for three times before using. Fish

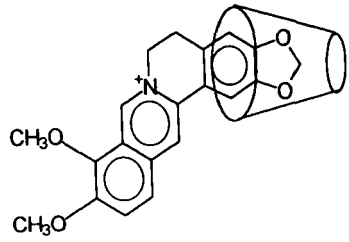

FIG.1. The binding of berberine cation to β -CD

sperm DNA was purchased from Shanghai ChangYang Pharmaceutical Factory (China) and was purified by phenol extraction, as described in the literature[10].Purity of the final DNA preparation was checked by monitoring the absorption spectra and the ratio of the absorbance at 260nm to 280nm. β -CD(A.R.) was the products of SuZhou Monosodium Glutamate Factory (China) and was recrystallized for two times in our laboratory. All experiments were performed in 0.02M TRIS buffer at room temperature, the deionized water was used.

RESULTS AND DISCUSSION

1. Binding of berberine with β -CD

FIG.2 shows the fluorescence spectra of berberine in the case of different concentrations of β -CD. That the intensity of fluorescence

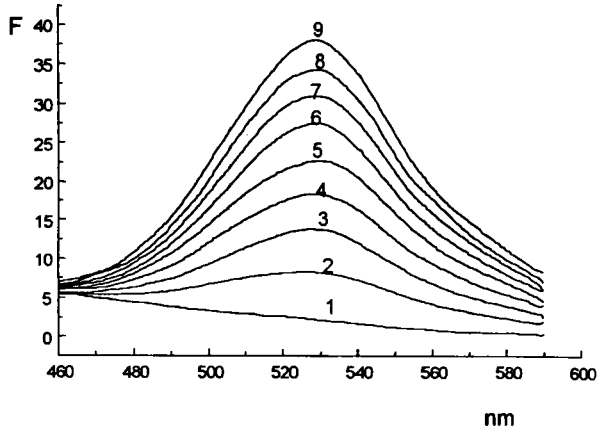

FIG.2. The fluorescence spectra of berberine, $C_{berberine}=1.0\times10^{-5}$mol/L, ①$C_{\beta\text{-CD}}$=0.0mol/L ②$C_{\beta\text{-CD}}=5.0\times10^{-4}$mol/L ③$C_{\beta\text{-CD}}=1.0\times10^{-3}$mol/L ④$C_{\beta\text{-CD}}=1.5\times10^{-3}$ mol/L ⑤$C_{\beta\text{-CD}}=2.0\times10^{-3}$mol/L ⑥$C_{\beta\text{-CD}}=2.5\times10^{-3}$mol/L ⑦$C_{\beta\text{-CD}}=3.0\times10^{-3}$mol/L ⑧$C_{\beta\text{-CD}}=3.5\times10^{-3}$mol/L ⑨$C_{\beta\text{-CD}}=4.0\times10^{-3}$mol/L

spectra of berberine enhances with the increasing of β-CD in the solution indicates that β-CD can include berberine cation and form an inclusion complex. The cavity of β-CD provides berberine a hydrophobic environment which accounts for the enhancement of berberine fluorescence spectra .When β-CD was added into the solution , the absorption spectra of berberine became higher, as is shown in FIG.3. But the change is little and there are little bathochromical shifts over the two absorption peaks at 227nm and 262nm, which reveals that the electronic condition of berberine doesn't change much. There isn't any shift over the peaks at 345nm

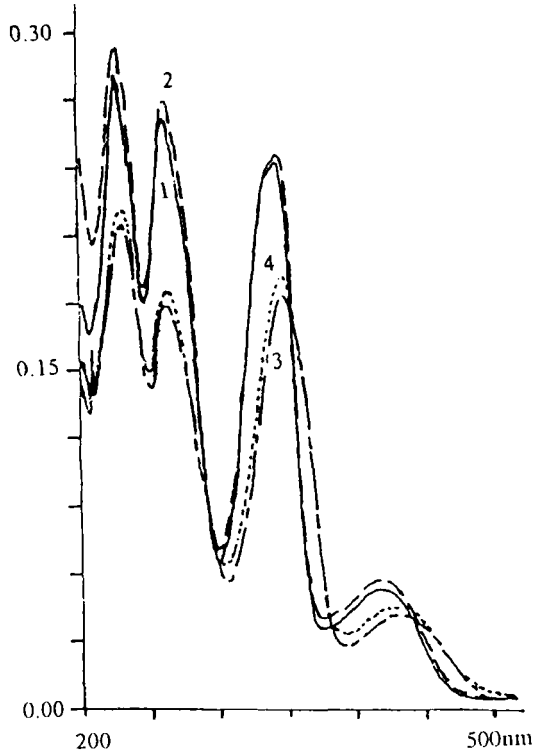

FIG.3 The absorption spectra of berberine (1.0×10^{-5} mol/L): (1) $C_{\beta\text{-CD}}$=0.0mol/L, C_{DNA}=0.0mol/L; (2) $C_{\beta\text{-CD}}$=0.0mol/L, $C_{DNA}=2.5\times10^{-4}$mol/L; (3) $C_{\beta\text{-CD}}=4.0\times10^{-3}$mol/L, C_{DNA}=0.0mol/L; (4) $C_{\beta\text{-CD}}=4.0\times10^{-3}$mol/L, $C_{DNA}=2.5\times10^{-4}$mol/L

and 418nm. It has been found that when berberine was in non-protonic solvents such as ethanol, the four absorption peaks of it bathochromic shifts.[11] The microenvironment provided by ethanol was similar to the one provided by the cavity of β-CD. The change of microenvironments around the isoquinolinic part may lead to great

change of the absorption spectra while the one around benzeniod part don't.[12] So it is the benzeniod part of berberine that was included into the cavity of β-CD, and the isoquinolinic part of berberine still exposes in water. The determination of association constants using spectroscopic measurements such as absorbance or fluorescence techniques is commonly accomplished by the method of Benesi-Hildebrand[12,13]. This analysis requires that the concentration of one of the associating species be kept very much lower than the other, and it assumes that the dissociated species do not contribute significantly to the measurement analytical signal (i.e.,<5%), the experimental conditions are carefully selected to meet the Benesi-Hildebrand relation:

$$C_B/F_{B-\beta-CD}=(Kk_iQ_{B-\beta-CD}C_{\beta-CD})^{-1}+(k_iQ_{B-\beta-CD})^{-1} \quad (1)$$

where C_B ,$C_{\beta-CD}$ are the concentrations of berberine and β-CD respectively and F is the fluorescence intensity of the complex. $Q_{B-\beta-CD}$ is the quantum efficiency of the complex and k_i is instrumental constant. According to FIG.2, using a double-reciprocal plot of $(C_B/F_{B-\beta-CD})$ versus $(1/C_{\beta-CD})$, the association constant K is determined from the ratio of the intercept to the slope, which is 2.8×

10^2 L/mol. The cavity of β-CD provides a high electron density microenvironment to berberine and thus it results in the increasing of the fluorescence quantum yield of berberine. The inclusion complex is a useful tool for us to investigate the interaction of berberine with DNA.

2. The interaction of berberine with DNA

Maiti and his coworkers have done much on this subject and they had found that berberine had great binding affinity with DNA.[7-9] Little attention was paid to the small molecule itself. It is important and necessary to know which part or functional group interacts with DNA in order to synthesis more efficient drugs.

By comparing the four curves in FIG.3, we can find that DNA changes all the four absorption peaks sharply. The bathochromic shifts of the two peaks at 418nm and 345nm, as well as the three isobestic points at about 442nm, 380nm and 354nm indicate that berberine has intercalated into the DNA double helix, as has been reported in literature[7,8] Although β-CD doesn't change the interaction of berberine with DNA much, the small difference between curve 3 and 4 in FIG.3 reveals that the included berberine by β-CD can still

intercalate into DNA. It shows that the part of berberine included in the cavity of β-CD is different from the one intercalating in DNA.

The absorption peaks at 419nm and 343nm corresponding to the energy separations L_a and L_b are related to the isoquinolinic part.[14] The bathochromic shifts of L_a and L_b band reveals that it is the isoquinolinic part of berberine that intercalates into the DNA double helix. On the other hand, the two peaks at 227nm and 262nm corresponding to the energy separations B_a and B_b are related with the benzenoid part of berberine. FIG.3 shows that the influence of β-CD over B_a and B_b bands is much more than it over L_a and L_b bands. So it is the benzenoid part that is included in the cavity of β-CD.

FIG.4 and FIG.5 is the absorption spectra of a series of solutions with various concentrations of DNA but with a constant concentration of berberine in the presence of β-CD and in the absence of β-CD, respectively. The intrinsic binding constant K was determined from the plot of $D/\triangle \varepsilon_{ap}$ versus D, where D is the concentration of DNA in base pairs, $\triangle \varepsilon_{ap}=[\varepsilon_a - \varepsilon_F]$ and $\triangle \varepsilon=[\varepsilon_B - \varepsilon_F]$. The apparent extinction coefficient, ε_a, is obtained by calculating A_{obsd}/[berberine]. ε_B and ε_F correspond to the extinction coefficient of the bound form

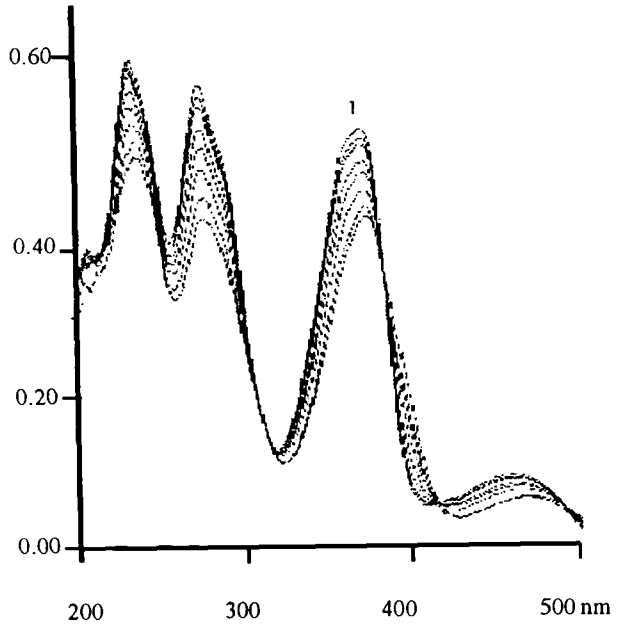

FIG.4 The absorption spectrum of berberine (2.0×10^{-5}mol/l) in the absence (1) and presence of increasing amounts of DNA.

of berberine and the extinction coefficient of free berberine. The data were fitted to equation 2, and K was obtained from the ratio of the slope to the y-intercept.

$$D/\triangle \varepsilon_{ap} = D/\triangle \varepsilon + 1/\triangle \varepsilon K \quad (2)$$

It was obtained the value of the intrinsic binding constant K was 4.5×10^{3}L/mol in the presence of β-CD and 1.1×10^{4}L/mol when β-CD is absent. The intrinsic binding constant K decrease sharply

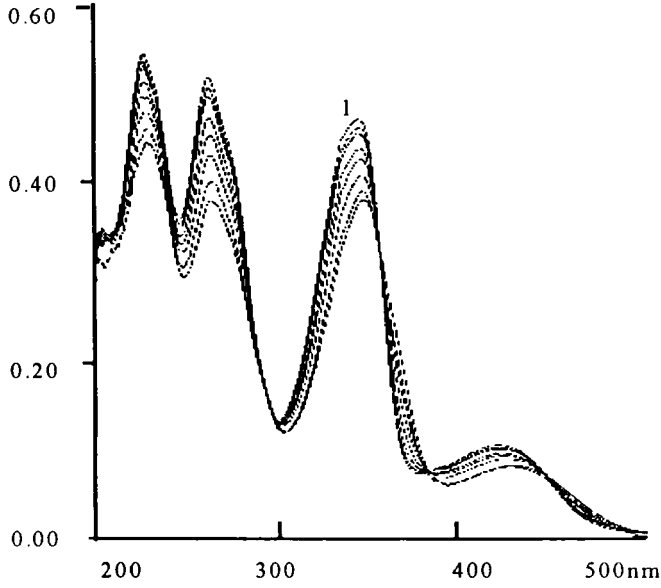

FIG.5 The absorption spectrum of berberine (2.0×10^{-5}mol/l) in the absence (1) and increasing amounts of DNA with β-CD (1×10^{-2}mol/l) in the solutions.

when β-CD exists. The great difference between the two values also indicates that β-CD still includes the berberine cation intercalating into the DNA double helix. If the part of berberine included by β-CD is the one which intercalates into DNA, the intrinsic binding constant K shouldn't change. But if β-CD and DNA can interact with different parts berberine at the same time, the change of K is reasonable. The several isobestic points manifest that berberine can

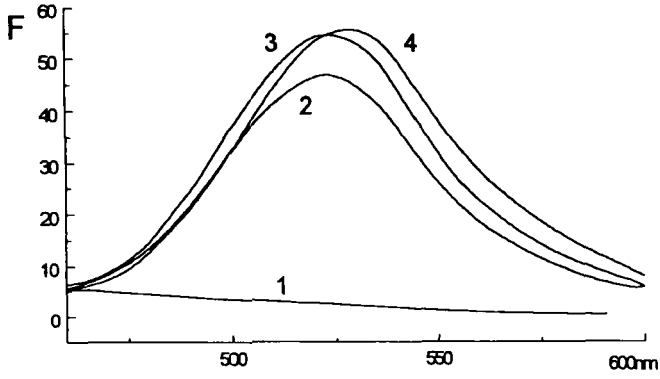

FIG.6. The fluorescence spectra of berberine, $C_{berberine}=1.0\times10^{-5}$mol/L, ①$C_{DNA}=0.0$mol/L, $C_{\beta\text{-}CD}=0.0$mol/L; ②$C_{DNA}=5.0\times10^{-4}$mol/L, $C_{\beta\text{-}CD}=0.0$mol/L; ③$C_{DNA}=0.0$mol/L, $C_{\beta\text{-}CD}=1.0\times10^{-2}$mol/L; ④$C_{DNA}=5.0\times10^{-4}$mol/L, $C_{\beta\text{-}CD}=1.0\times10^{-2}$mol/L.

intercalate into the DNA double helix and form a new substance with the existence of β-CD.

The fluorescence experiments also support the conclusion drawn above. FIG.6 shows that both β-CD and DNA can enhance the fluorescence intensity of berberine. But the maximum emission in the case of β-CD is at 529nm while at 524nm in the case of DNA and the influence of DNA over the fluorescence of berberine is larger than that of β-CD. The energetic difference between the excited state and the ground state of berberine, determining the position of the fluorescence bands, can be influenced by the microenvironments of

both isoquinolinic part and the benzenoid part.[14] The fluorescence intensity is much larger when β -CD and DNA coexist in the solution. And the fact shows that β -CD and DNA can interact with berberine at the same time.

CONCLUSION

In the presence of β -CD, we studied the interaction of berberine more clearly and found it was the isoquinolinic part of berberine that intercalated into the DNA double helix. We hope the new method be useful for the study of binding of small molecules with large biomolecules.

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