1054 Chinese Chemical Letters Vol. 14, No. 10, pp1054– 1057,2003
http://www.imm.ac.cn/journal/ccl.html

Interaction of Hypocrellin B or Mono-cysteine Substituted Hypocrellin B with CT-DNA by Spectral Methods

Jia Hong ZHOU, Sheng Qin XIA,Xue Song WANG*, Bao Wen ZHANG*, Yi CAO

Technical Institute of Physics and Chemistry, Chinese Academy of Sciences,Beijing 100101

Abstract: The interaction of the anticancer drug hypocrellin B (HB) or the mono-cysteine substituted hypocrellin B (MCHB) and calf thymus deoxyribonucleic acid (CT-DNA) has been investigated using spectral methods. The results of UV-visible spectra show that the HB and MCHB could intercalate into the base-stacking domain of the CT-DNA double helix. The studies of fluorescence spectra and circular dichroism(CD) spectra also support the interacalation mechanism.

Keywords: Hypocrellin B, mono-cysteine substituted hypocrellin B, CT-DNA, interacalation mechanism.

Hypocrellins are perylene- quinonoid pigments isolated from *Hypocrella bambusae*[1] in China. They have been used as a phototherapeutic agent against various skin diseases and superficial tumors[2]. Recently, investigations show that Hypocrellins possess a light-induced toxicity against the human immunodeficiency virus (HIV-1), the vesicular stomatitis virus (VSV), and the tumor [3]. In the phototherapeutic treatment of virus or tumor cells, one possible target of hypocrellins is DNA molecule. It has been reported that hypocrellin A (HA) and hypocrellin B (HB) can result in the photodamage of plasmid pBR322 DNA[4] and calf thymus deoxyribonucleic acid (CT-DNA) [5], respectively. The photodynamic properties of hypocrellins depend strongly on the way by which ground state hypocrellins interact with DNA target (*e.g.* intercalation, surface binding, or no affinity at all). However, to our knowledge, no direct study on the interaction of ground state hypocrellins and DNA is reported until now. The research on the direct interaction occurring between hypocrellins and DNA would be helpful in understanding the therapeutic mechanism of hypocrellins and their ramifications in depth, and in designing and synthesizing new ramifications which have stronger binding ability to DNA and possess higher phototherapeutic effect on DNA.

In this letter, the interaction mechanism between CT-DNA and HB or MCHB (**Figure 1**) was studied by spectral methods, the results indicate that the HB and MCHB can intercalate into the base pairs of CT-DNA molecule.

* E-mail: g203@ipc.ac.cn

Results and Discussion

HB and MCHB were prepared according to the literatures [6]. The aqueous solutions of them were prepared by adding small amounts of concentrated DMSO solutions of HB or

Figure 1 The structures of HB (left) and MCHB (right)

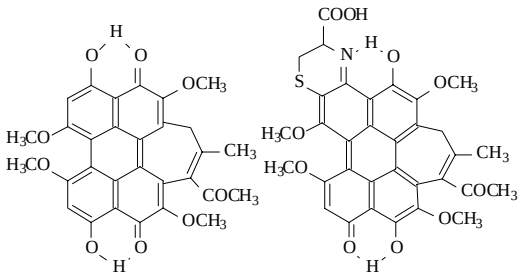
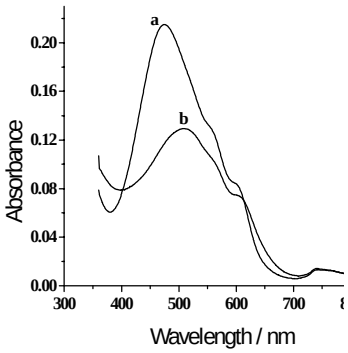


Figure 2 The change of UV-visible absorption spectrum of HB aqueous solution (0.1mol/L) upon addition of CT-DNA.



MCHB to double distilled water [7]. In the region of 360 nm to 800 nm of the UV-visible absorption spectrum, HB has a characteristic absorption band with maximum at 466 nm due to π-π* transition (curve a in **Figure 2**). Upon addition of increasing amounts of CT-DNA to a series of aqueous solution containing a fixed concentration of HB, notable changes in the UV-visible absorption spectrum of HB were observed after mixing for 12 hours in dark. The absorption bands at 466 nm shifted to longer wavelength, and the spectrum showed strong decrease in the peak intensities (hypochromicity) on increasing the concentration of CT-DNA. When 120 uL CT-DNA was added, the peak at 466 nm shifted to 535 nm (curve b in **Figure 2**), and the intensity decreased 34%. It is known that intensity changes in the absorption band (hypochromism) and shift in the wavelength (red shift, bathochromism) are a hallmark for intercalation of chromophores to DNA helix[8]. Therefore, Such pronounced hypochromism and bathochromism observed from the absorption spectrum of HB suggest there is a strong intercalation of HB molecule into CT-DNA base pairs. This implies a close proximity of HB chromophore and the CT-DNA base pairs, *i.e.*, a strong overlap between the electronic states of the intercalating chromophore and those of the CT-DNA bases occurs. It is different from the above results of HB, MCHB has two characteristic absorption bands at 591 and 753 nm (curve a in **Figure 3**) [6], respectively. The short wavelength band (591 nm) comes from π-π* transition of HB moiety, while the long wavelength band (753 nm) could be assigned to be the intramoleculer charge transfer (ICT) transition from the N and S atoms of the cysteine moiety to the π* orbital of HB moiety[6]. When adding 120 uL CT-DNA into the solution of MCHB, the absorption band at 591 nm shifted to 606nm, and the intensity decreased 24%(curve b in **Figure 3**), suggesting MCHB also interacts with CT-DNA in the same way as that of HB, but the binding ability of MCHB to CT-DNA is smaller than that of HB. The enhancement of steric hindrance for MCHB approaching

to CT-DNA due to the substitute of cysteine may account for the weak binding ability of MCHB to CT-DNA in comparing with HB. On the other hand, the phenomenon that the absorption band at 753 nm decreased drastically in intensity indicates that the ICT transition is forbidden, which might result from the conformation changes of MCHB caused by intercalation of MCHB into the base pairs of CT-DNA. The conformation changes might reduce the overlap of the lone-pair electrons on N and S atoms with the π^* orbital of HB moiety, and as a result, weaken the ICT transition significantly.

The binding of HB to CT-DNA was also studied by fluorescence spectroscopy. The fluorescence intensity of HB was weak in aqueous solutions, but the addition of CT-DNA caused a gradual increase of the fluorescence intensity and a red shift of emission maximum. Because HB molecules were bound to CT-DNA in a relatively non-polar environment compared to water; and the collisional frequency of the solvent molecules with HB molecules decreased due to planar aromatic backbone of HB molecule has stacked between adjacent base pairs of CT-DNA[9]. There is a similar phenomenon in the case of MCHB.

To further confirm binding interactions, ethidium bromide (EB) fluorescence displacement experiments were carried out. EB is the most widely used fluorescence probe for DNA structure and has recently been employed to examine the mode and process of drug binding to DNA[10]. When the system of the CT-DNA-EB was titrated with HB or MCHB, the emission intensity of the CT-DNA-EB system decreased as the concentration of titer increased with the addition of HB or MCHB. The changes observed here are often characteristic of intercalation process[11]. This phenomenon indicate that titer replaces EB from the CT-DNA-EB system and the replacement leads to the decrease in emission intensity of the CT-DNA-EB system. The addition of HB gave rise to more decrease of the CT-DNA-EB fluorescence emission intensity than the addition of the same amount of MCHB, which is consistent with the results of UV-visible spectra, and indicate that the binding ability of HB to CT-DNA is larger than

Figure 3 The UV-visible absorption spectrum changes of MCHB aqueous solution (0.1mmol/L) upon addition of CT-DNA.

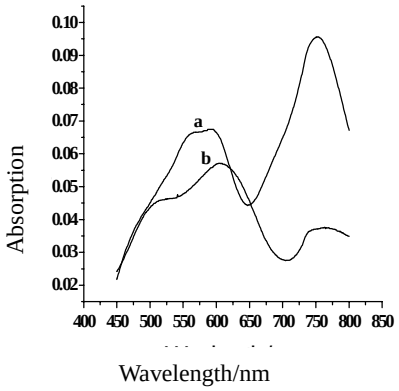
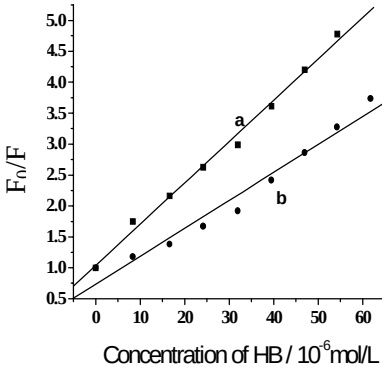


Figure 4 Emission quenching of CT-DNA EB system by HB (curve a) or MCHB (curve b)



that of MCHB. Plotting F_0 / F *versus* HB (curve a) or MCHB (curve b) gives a straight line, where F_0 / F represents the ratio of emission intensity in the absence and presence of [HB] or [MCHB], with linear correlation coefficient of 0.997 and 0.984, respectively (**Figure4**). The results suggest that only one kind of quenching process is involved and that HB or MCHB bind to CT-DNA mainly by one mode, *i.e.*, intercalation[12].

Circular Dichroism (CD) spectrum measurement of the DNA molecules is one of the effective methods to monitor the conformational changes brought about by the interaction of host and guest molecules[13]. The CD spectrum of CT-DNA exhibited a positive absorption band at 275 nm due to the base stacking and a negative band at 245 nm due to the helicity of B-DNA[14]. When HB or MCHB was added, both intensities for the positive and negative bands decreased significantly. It suggested that the helicity and the extent of stacking among DNA base pairs have been disturbed due to the intercalation of HB or MCHB. The effect of MCHB on the helicity and the extent of stacking among DNA base pairs were smaller than HB, indicating the binding ability of HB is larger than that of MCHB once again.

In summary, the direct interaction occurring between HB or MCHB and DNA makes the characteristic absorption bands of HB or MCHB have the pronounced hypochromism and bathochromism, which indicates that HB and MCHB could intercalate into the base pairs of CT-DNA molecule. Further evidences for the intercalation mechanism were obtained from fluorescence spectroscopy experiments, ethidium bromide fluorescence displacement studies and Circular Dichroism measurements. The study on the interaction between HB or MCHB and CT-DNA is under way of time-resolved fluorescence spectra, gel electrophoresis and resonance Raman spectra.

Acknowledgment

This work was financially supported by NNSFC

References

1. X. Y. Wan, Y.T.Chen, *Chin. Sci. Bull.*, **1980**, *24*,1148.
2. Z.Y. Liang, G. D. Mei, W. Y. Zhu, *et al*, *Chin. J. Dermatol.*, **1982**,*15*,87.
3. Z. J. Wang, Y. Y. He, C. G. Huang, *Photochem. Photobiol.*,**1999**,*70*, 773.
4. W. Zou, J. Y .An, L. J. Jiang, *J. Photochem. Photobiol. B: Biol.*, **1996**, *33*,73.
5. Z. Z. Ou, J. R. Chen, X. S. Wang,*et al*, *Chem. Lett.*, **2002**,206.
6. Y. Y. He, J. Y. An, L. J. Jiang, *J. Photochem. Photobiol. A:Chem.*, **1998**, *115*, 213.
7. G. Cervantes, S. Marchal, M. J. Prieto, *et al*, *J. Inorg. Biochem.*, **1999**,*77*,197.
8. V. A. Bloomfield, D. M. Crothers, I. Tinoco, in: Harper, Row (Eds.), *Physical Chemistry of Nucleic Acids*, New York, **1974**, p.432.
9. J. K. Barton, J. M. Goldberg, C. V. Kumar, N. J. Turro, *J.Am.Chem.Soc.*, **1986**,*108*,2081.
10. M.K.Pal, J.K.Ghosh, *Spectrochim. Acta A*, **1995**,*51*, 498.
11. C. V. Kumar, J. K. Barton, M. J. Turro, *J.Am.Chem.Soc.*, **1985**,*107*,5518.
12. Y. M. Song, J. W. Kang, J. Zhou, *et al*, *Spectrochim. Acta A*, **2000**,56,2491.
13. D. M. Gray, R. L. Ratliff, M. R. Vaughan, *Methods Enzymol.*, **1992**, *211*,389.
14. R. Amutha, V. Subramanian, and B. U. Nair, *Chem. Phys. Lett.*, **2001**,*344*,40.

Received 27 Septemer, 2002