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Interaction of Myricetin with ds-DNA Analyzed by Spectrophotometry and Cyclic Voltammetry Techniques

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Cyclic voltammetry coupled with UV/VIS spectroscopic techniques were used to study the interaction of myricetin, a flovonoid compound, with double stranded calf thymus DNA (ds-DNA) in phosphate buffer solution (pH 7.5). In spectrophotometric studies, maximum absorbance at *ca.* 330 nm for myricetin does not change significantly by addition of ds-DNA to the solution. The slopes of the calibration curves for methotrexate in the absence and presence of neutral red (NR)-DNA are similar. Because of the inactivity of myricetin on the surface of hanging mercury drop electrode, we used neutral red as a probe for electrochemical study of myricetin with ds-DNA. In the electrochemical study of neutral red, the appearance of a pair redox peaks with DEp = 3.0 mV shows the adsorption process. The decreasing of peak currents for neutral red by addition of ds-DNA reveals a high strength binding between neutral red and DNA (Kb = 2.74×10^4). In the competitive study on the titration of neutral red-DNA with myricetin shows no significant changes on the oxidation or reduction of neutral red-DNA. These studies are valuable addition for a better understanding of the detailed mode of interaction between myricetin with ds-DNA, which should be important in deeper insight into the therapeutic efficacy of myricetin and design of new DNA targeted drugs. All of the electrochemical and spectroscopy studies show that myricetin has a week interaction with ds-DNA.

Key Words: Myricetin, ds-DNA, Neutral red, Interaction, Flavonoids, Spectrophotometry, Cyclic voltammetry.

INTRODUCTION

Flavonoids are polyphenolic benzo-c-pyrone compounds that belong to a class of water soluble plant pigments. More than 6000 different flovonoid molecules have been identified. They occur in vegetables, fruits and beverages such as beer, wine, tea and fruit drinks[1].

Flavonoids show to possess both antioxidative and cytoprotective properties[2,3], has been applied to traditional Chinese medicines successfully for the treatment of depression and anxiety[4]. Myricetin (3,3´,4´,5,5´,7-hexahydroxy flavone) (Fig. 1), a naturally occurring flavonoid, is classified as a flovonoid with strong antioxidant effects. Oxidative stress plays a key role in various neurological diseases such as ischemia and Alzheimer's disease.

In the last decades, much attention was paid to the binding of small molecules with DNA, as a result of decided advantages of these molecules as potential drugs. Many natural or synthetic drugs serve as analogues in the research of protein-nucleic acid recognition and provide site-specific reagents for molecular biology. Therefore, the investigation of drug-DNA interaction is important for understanding the molecular mechanisms of the drug action and designing specific DNA-targeted drug[5]. Since the concept of intercalation into DNA was first formulated by Lerman[6], it has become widely recognized that many compounds of pharmacological interest, including anticancer drugs and antibiotics correlate their biological and therapeutic activities with the ability of intercalative interaction with DNA[7]. This noncovalent binding has an important function in life phenomena at the molecular level, deciding the interaction specificity of drug with DNA.

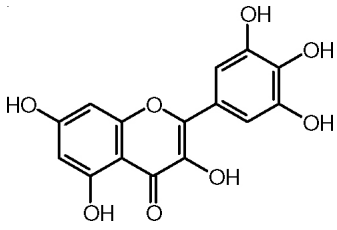

Fig. 1. Chemical structure of myricetin

Neutral red (NR, Fig. 2) is a planar phenazine dye and in general, is structurally similar to other planar dyes, *e.g.*, those of the acridine, thiazine and xanthene kind. In recent years, the interaction of the fluorescent neutral red dye with DNA has been demonstrated by spectrophotometric[8-10] and electrochemical[11] techniques. Compared with a common fluorimetric probe, ethidium bromide (EB)[12,13], the neutral red dye offers

lower toxicity, higher stability and convenience of use[14,15]. In addition, its solution remains stable for up to 2 years. We used neutral red as a probe to study on the competitive interaction of neutral red-DNA with myricetin.

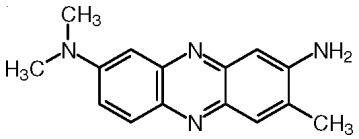

Fig. 2. Chemical structure of neutral red (NR)

To our best of knowledge there has not yet any report about the interaction of myricetin-DNA based on the electrochemical and especially spectroscopic characteristics. Accordingly, in this work, detailed investigations of the electrochemical behaviour of myricetin upon addition of ds-DNA were carried out. Moreover, the changes in the electronic absorption spectra, when myricetin binds to ds-DNA have investigated. The agreement of the various methods is quite good. Thus it can be seen, there is a mutual complement between electrochemical method and spectroscopy techniques, which can provide fruitful information about the mechanism of interaction and the conformation of adduct from different aspects.

EXPERIMENTAL

Deoxyribonucleic acid sodium salt from calf thymus (Sigma Chem. Co., USA) was used without further purification and its stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water and stored at 4 ºC. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient ε_{260} = 6600 L mol^{-1} cm^{-1}. Purity of the DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of > 1.8 at A_{260}/A_{280}, which indicates that DNA was sufficiently free from protein[8]. Myricetin stock solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving its powder (Sigma Chem. Co., USA) in deionized water and stored in a cool and dark place. Phosphate buffer (20 mmol L^{-1}, pH 7.5) and potassium chloride (KCl) for adjusting ionic strength were purchased from Fluka (USA). Neutral red dye stock solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving its crystals (Sigma-Aldrich) in water and diluted to the required volume.

The spectra in the wavelength range of 200-700 nm was recorded by a Shimadzo model 1650 UV-VIS spectrophotometer. Voltammetric measurements were carried out using a Metrohm instrument, model 797 VA, computrace with stand three-electrodes containing a hanging mercury drop as a working electrode, a carbon rod as an auxiliary electrode and a Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode.

RESULTS AND DISCUSSION

Electronic absorption spectra of myricetin in the presence of DNA: UV-Visible spectroscopy is the most common and convenient way to study the interaction between small molecules or rare earth complexes with nucleic acid. Molecules containing aromatic or phosphate chromophore groups can interact with double helix structure of DNA, therefore, the interaction between them can be studied according to changes in the absorption spectra before and after reaction. Red shift (or blue shift), hyperchromic (or hypochromic) effects and isochromatic point are spectral properties of DNA-drug interaction, which closely related with the double helix structure[16]. Generally, red shift (or blue shift) and hypochromic (or hyperchromic) effect are observed in the absorption spectra if molecules intercalate with DNA. Hypochromic effect is obvious if the intercalation is strong[17,18].

In this work, UV-VIS spectrophotometric titration of a solution containing myricetin with (1.0×10^{-5} mol L^{-1}) was recorded by ds-DNA solution. The results (Fig. 3) show that three absorption bands at 228, 270 and 330 nm for myricetin in the absence of DNA. The absorbance at 330 nm does not change significantly by increasing the concentration of ds-DNA.

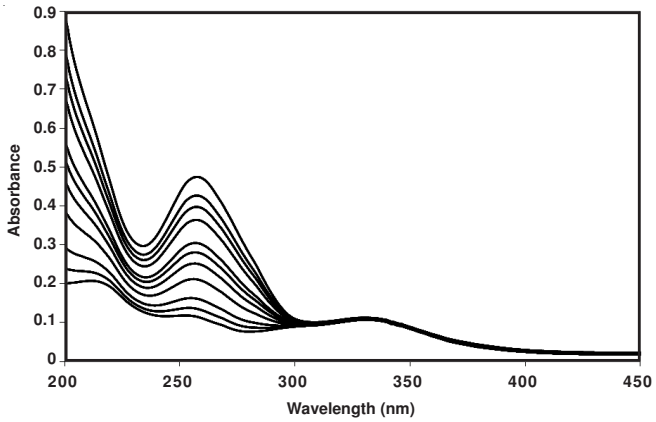

Fig. 3. Absorption spectra of myricetin in different concentrations of ds-DNA. C_{DNA} = 0.0, 6.1, 12.2, 18.3, 24.4, 30.5, 36.6, 42.7, 48.8, 54.9 and 61.0 μmol L^{-1} for curves 1-11 and $C_{myricetin}$ = 10.0 μmol L^{-1} in phosphate buffer (0.002 mol L^{-1}, pH 7.5)

Interaction of neutral red with ds-DNA by spectrophotometry titration: Fig. 4 shows the absorption spectra of the neutral red with the addition of DNA. It can be seen that the absorption peak of the neutral red at about 455 nm exhibited gradual decrease and a slight red shift with the increasing concentration of DNA and a new band at about 545 nm appeared. An isobestic point at 490 nm provided evidence of the new DNA-neutral red complex formation. We calculated the binding constant between neutral red-DNA according to double-reciprocal equation[19]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \times \frac{1}{K[DNA]} \quad (1)$$

where, A_0 and A are the absorbances of CPT-11 in the absence and presence of DNA, respectively and ε_G and ε_{H-G} are their absorption coefficients. The double reciprocal plot of $\frac{A_0}{A - A_0}$ *versus* $\frac{1}{[DNA]}$ was linear and the binding constant was calculated as 2.74×10^4 mol^{-1} L from the ratio of the intercept to the slope equation (Fig. 5). Neutral red can intercalate into the base pairs of double helix DNA uniquely[20], so it was employed as molecule probe in the study.

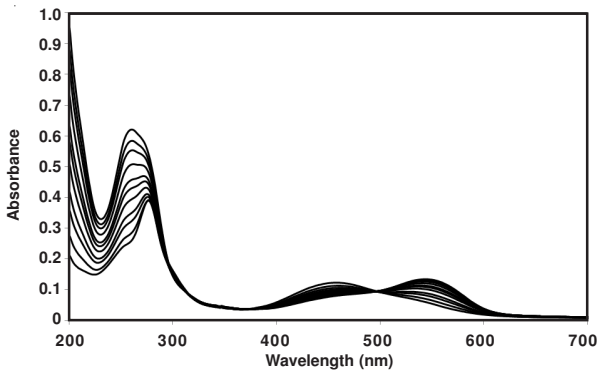

Fig. 4. Absorption spectra of neutral red in different concentrations of ds-DNA. C_{DNA} = 0.0, 6.7, 13.4, 20.1, 26.8, 33.5, 40.2, 42.7, 46.9, 53.6, 60.3 and 67.0 μmol L^{-1} for curves 1-11 and C_{NR} = 10.0 μmol L^{-1} in phosphate buffer (0.002 mol L^{-1}, pH 7.5)

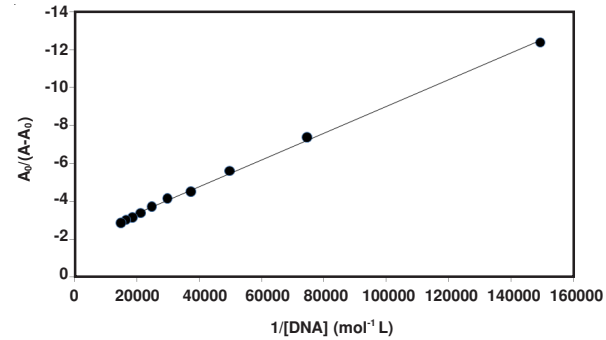

Fig. 5. Plot of $A_0/(A-A_0)$ *versus* 1/[DNA] for neutral red-DNA system at 456 nm. All other conditions are similar to Fig. 4

Competitive interaction of myricetin with neutral red-DNA: A competitive interaction between myricetin and neutral red-DNA system was studied by the addition of myricetin to a solution containing neutral red and DNA. As it is clearly shown in Fig. 6 the spectrum of neutral red-DNA has two maximum absorbances at 285 and 500 nm. The absorbance at 285 nm increases by addition of myricetin and a new peak appeared at 330 nm. The absorbances in the wavelength range of 500-600 nm do not change significantly because of the stronger binding constant of neutral red-DNA in comparison with myricetin-DNA system. Also the slopes of calibration curves for myricetin at 330 nm in the absence and presence of neutral red-DNA do not differ significantly (Fig. 7).

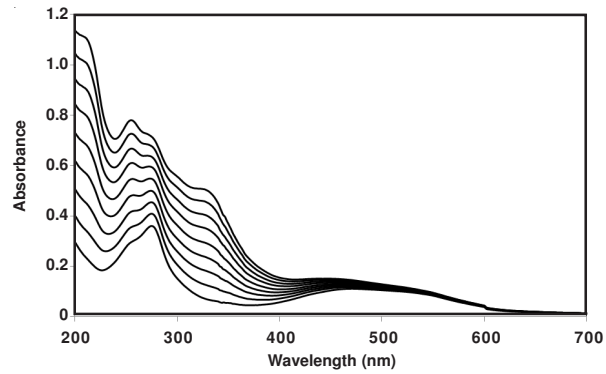

Fig. 6. Absorption spectra of the competitive interaction between myricetin and neutral red bonded to ds-DNA at $C_{myricetin}$ = 0.0, 6.7, 13.4, 20.1, 26.8, 33.5, 40.2, 42.7, 46.9 and 53.6 μmol L^{-1} for curves 1-10, C_{NR} = 10.0 μmol L^{-1} and C_{DNA} = 30.5 μmol L^{-1} in phosphate buffer (0.002 mol L^{-1}, pH 7.5)

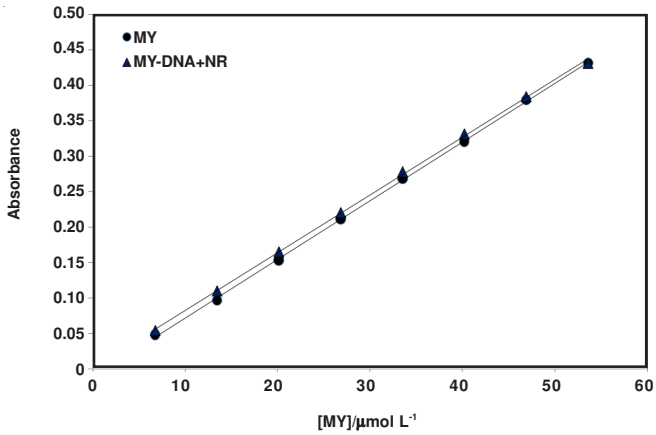

Fig. 7. Calibration curves for myricetin in the absence and presence of neutral red-DNA at 330 nm. Conditions: phosphate buffer (0.002 mol L^{-1}, pH 7.5), C_{NR} = 10.0 μmol L^{-1}, C_{DNA} = 18.3 μmol L^{-1}

According to the above observations, it seems that no exchange take place between myricetin and neutral red-DNA adduct. In addition unchanging in position of spectral band at 330 nm in the absence and presence of DNA suggests that myricetin has a week interaction with double helix of DNA.

Electrochemical studies: Because of the inactivity of myricetin on the surfaces of hanging mercury drop electrode, silver and gold electrodes, we used neutral red as an electrochemical probe for the study on the interaction of myricetin with ds-DNA. Neutral red has a totally reversible electrochemical reaction on hanging mercury drop electrode.

Electrochemical behaviour of neutral red-DNA on the surface of hanging mercury drop electrode: The electrochemical behaviour of neutral red in absence and presence of ds-DNA at hanging mercury drop electrode was studied by cyclic voltammetry. The appearance of a reduction peak at - 0.584 V (*vs.* Ag/AgCl) and an oxidation peak at -0.572 V in pH 7.5 phosphate buffer revealed the adsorption of neutral red on the hanging mercury drop electrode because the ΔE_p is about 12 mV (Fig. 8). The addition of DNA into the neutral red solution caused a considerable diminish at peak currents as shown in Fig. 8. The binding of neutral red to ds-DNA should lead to a significant decrease of peak current due to the formation of neutral red-DNA adduct with very small diffusion coefficient[21-23].

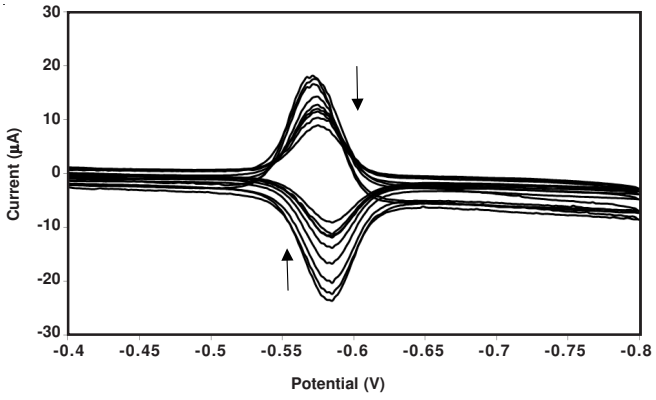

Fig. 8. Cyclic voltammetry of neutral red at different concentrations of ds-DNA on the surface of hanging mercury drop electrode. Conditions: C_{DNA} = 0.0, 4.6, 9.3, 14.0, 18.8, 23.6, 28.4, 33.3 and 38.3 μmol L^{-1}, $C_{myricetin}$ = 5.0 μmol L^{-1}, n = 50.0 mV s^{-1}, pulse amplitude 2.0 mV, phosphate buffer (0.002 mol L^{-1}, pH 7.5), repetition No. 2

Tafel plot analysis: In order to determine the apparent transfer coefficients 'a' and the number of electron transferred 'n' of neutral red in absence and presence of ds-DNA, the cathodic Tafel plots of neutral red and neutral red-DNA on hanging mercury drop electrode was plotted using the cyclic voltammetry. By linear regression fit of the cathodic Tafel plots, cathodic Tafel slopes were determined which were 21.731 V Dec^{-1} and 24.484 V dec^{-1} in the absence and presence of ds-DNA respectively. Thus, the values of αn_a were calculated as 1.28 and 1.45 for reduction peaks of neutral red and neutral red-DNA adduct, respectively.

Competitive electrochemical interaction of myricetin with neutral red-DNA: Further support for the mode of binding between myricetin and ds-DNA is given through the competitive interaction of myricetin and neutral red with DNA. The reduction peak of DNA-neutral red at hanging mercury drop electrode does not change significantly with the increasing concentration of myricetin (Fig. 9). According to the above observations, it is suggested that the myricetin do not have strong interaction with ds-DNA and can't substitute with neutral red in the DNA-neutral red system.

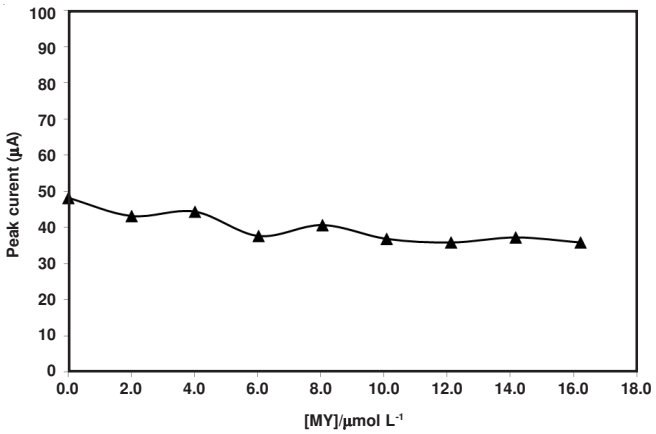

Fig. 9. Dependence of cathodic peak current of neutral red-DNA complex at different concentrations of myricetin. Cmyricetin= 0.0, 2.0, 4.0, 6.0, 8.0, 10.1, 12.1, 14.2 and 16.2 µmol L-1 for curves 1-9, C_{NR} = 5.0 µmol L-1 and C_{DNA} = 18.3 µmol L-1 in phosphate buffer (0.002 mol L-1, pH 7.5), n = 50.0 mV s-1, pulse amplitude 2.0 mV

Conclusion

In this work, the interaction of myricetin with ds-DNA was studied by cyclic voltammetry. The binding of neutral red as a probe to ds-DNA resulted in a series of changes in the electrochemical behaviour and spectral characteristics. Upon binding to ds-DNA, the absorptivity coefficient of neutral red showed peculiar changes and the oxidation and reduction peak currents were efficiently decreased. From these experimental results, it could be affirmed that the interaction of neutral red with ds-DNA is through intercalative mode. In contrast, myricetin has low affinity for the ds-DNA base pairs. The electrostatic repulsion between the anionic charge on myricetin at pH 7.5 and the ds-DNA phosphates is not expected to improve the DNA binding affinity. These investigations showed that cyclic voltammetry coupled with intercalative probes could provide a convenient way to characterize both the binding mode and the interaction mechanism of small molecules with DNA, which is important for the design of new pharmaceuticals.

ACKNOWLEDGEMENTS

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REFERENCES

1. H.A. Scheidt, A. Pampel, L. Nissler, R. Gebhardt and D. Huster, *Biochim. Biophys. Acta*, **1663**, 97 (2004).
2. F. Dajas, F. Rivera, F. Blasina, F. Arredondo, C. Echeverry, L. Lafon, A. Morquio and H. Heizen, *Neurotox. Res*., **5**, 425 (2003).
3. L. Mira, M.T. Fernandez, M. Santos and R. Rocha, *Free Radic. Res.*, **36**, 1199 (2002).
4. I.M. Liu, S.S. Liou and J.T. Cheng, *J. Ethnopharmacol.*, **104**, 199 (2006).
5. R.K. Gilpin, *Anal. Chem.*, **69**, 145 (1997).
6. L.S. Lerman, *J. Mol. Biol*., **3**, 18 (1961).
7. M.J. Waring, The Molecular Basis of Antibiotic Action, John Wiley and Sons, New York, United States, p. 173 (1972).
8. S. Mahadevan and M. Palaniandavar, *Inorg. Chem.*, **37**, 693 (1998).
9. Y.T. Wang, F.L. Zhao, K.A. Li and S.Y. Tong, *Anal. Chim. Acta*, **396**, 75 (1999).
10. C.Z. Huang, Y.F. Li and P. Feng, *Talanta*, **55**, 321 (2001).
11. Z.X. Wang, Z.L. Zhang, D.J. Liu, S.J. Dong, *Spectrochim. Acta A*, **59**, 949 (2003).
12. H. Heli, S.Z. Bathaie and M.F. Mousavi, *Electrochem. Commun.*, **6**, 1114 (2004).
13. J.B. Lepecq and C. Paoletti, *J. Mol. Biol*., **27**, 87 (1967).
14. V.W.F. Burns, *Arch. Biochem. Biophys.*, **145**, 248 (1971).
15. Y. Cao, Y.J. Li and X.W. He, *Chem. J. Chin. U.*, **20**, 709 (1999).
16. P. Yang and C.Q. Zhou, *Acta Chim. Sinica*, **61**, 1455 (2003).
17. S.A. Ysoe, A.D. Baker and T.C. Strekas, *J. Phys. Chem.*, **97**, 1707 (1993).
18. E.J. Gao, S.M. Zhao, Q.T. Liu and R. Xu, *Acta Chim. Sinica*, **62**, 593 (2004).
19. M. Purcell, J.F. Neault and T. Riahi, *Biochim. Biophys. Acta*, **1478**, 61 (2000).
20. Y.N. Ni, D.Q. Lin and S. Kokot, *Talanta*, **65**, 1295 (2005).
21. I.S. Shehatta and M.S. Ibrahim, *Can. J. Chem.*, **79**, 1431 (2001).
22. M. Aslanoglu and G. Ayne, *Anal. Bioanal. Chem.*, **380**, 658 (2004).
23. R. Hajian, N. Shams and N. Azarinezhad, *Asian. J. Chem.*, **22**, 8007 (2010).