Int. J. Electrochem. Sci., 6 **(2011)** 1669 - 1678

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Investigation of the Electrochemical Interaction Behavior of DNA with 5-Fluorouracil Derivatives

Ke-jun Zhang[1, 2], *Wu-yi Liu*[1, *]

[1] Department of Biology Science, Fuyang Normal College, Fuyang City 236041, China
[2] Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao City 266071, China
[*]E-mail: lwycau@yahoo.com.cn

Received: 31 March 2011 / *Accepted:* 9 April 2011 / *Published:* 1 May 2011

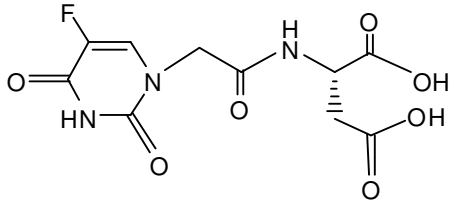
Although many 5-Fluorouracil derivatives have been synthesized and identified, there are few reports on the interaction modes of different anti-carcinogen 5-Fluorouracil derivatives with Deoxyribonucleic acid (DNA). In this study, five new anti-carcinogen 5-Fluorouracil derivatives with DNA were investigated, i.e. 5-FU-Asp, 5-Fu-Trp, 5-FU-Ser, 5-FU-Tyr, and 5-FU-Phe, and their DNA binding specificities were compared by electrochemical means with previous reported DNA ligands. Meanwhile, the cyclic voltammetry has been proved to be powerful for the elucidation of interactions between DNA and 5-Fluorouracil derivatives, with $Fe(CN)_6^{3-/4-}$ as electroactive indicator of the intercalative interaction dominance. Using this electrochemical approach and Deoxyribonucleic acid (DNA) modified gold electrodes prepared by the dry adsorptive method, the electrochemical behaviors and modes of 5-Fluorouracil derivatives targeting DNA were well studied. The binding of 5-Fluorouracil derivatives with DNA, analyzed in terms of the cooperative Hill model, yields different association constants and binding numbers. This study serves as a good reference for synthesis, structural characterization of novel 5-Fluorouracil derivatives.

Keywords: DNA, anti-carcinogen, derivative of 5-Fluorouracil, intercalation, cyclic voltammetry

1. INTRODUCTION

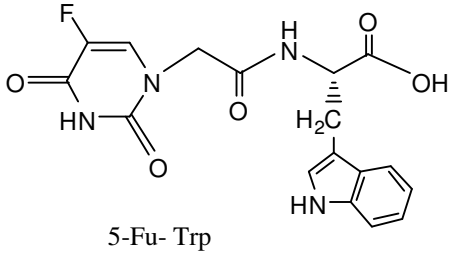
Analysis of DNA interaction with small molecules such as drugs, organic dyes and metals, has been an intensive topic for decades, because it provides insight into the screening and design of novel and/or more efficient drugs targeting DNA, and could speed up the drug discovery and development processes [1]. Moreover, study on the properties of anti-carcinogenic medicines and their interaction with DNA is also significantly important in developing new cancer therapy treatments or anti-

carcinogens. The recognition of DNA binders involves a complex interplay of different interactive forces. It includes hydrophobic interaction along the minor groove of DNA, strong electrostatic interaction arising from the exterior sugar-phosphate backbone and intercalative interaction between the stacked bases pairs of native DNA from the major grooves [2-4]. A variety of analytical techniques have been developed for characterization and identification of the interaction between DNA and small molecules with relative advantages and disadvantages [4-9]. However, most of these methods suffer from high cost, low sensitivity and procedural complication. Up to now, electrochemical methodologies have attracted appreciable attention due to the inherent specificity and high sensitivity. Direct monitoring, simplicity and low cost facilitate to investigate the drug-targeting compound interactions and obtain the quantitative analysis information in pharmaceutical formulations and biological fluids [10, 11]. On the other hand, the electrochemical system can serve as a versatile and illuminating model of biological system in a way to the real action occurring in the living cells *in vivo* [12, 13]. The interaction mechanism can at least be elucidated in three different ways, involving the use of drug- and/or DNA-modified electrodes and interaction in solution [13]. 5-Fluorouracil (5-FU) has been increasingly employed alone or in therapy combined with various cytotoxic drugs and hormones in the treatment of several tumors, such as breast, colorectal and gastric cancers [14-20]. However, because of the poor tumor selectivity and high incidence of toxicity in the bone marrow, gastrointestinal tract, central nervous system and skin, many derivatives of 5-FU have been developed to improve the topical delivery and reduce the side effects [21-26]. Aminophenol play important roles in the life status of human beings and other organisms; they function as hormone, enzyme inhibitor/substrate, growth promoter, inhibitor, neurotransmitter, immunomodulating agents as well as antibiotics, driving considerable pharmacological interest in design and application of novel drugs [27-30]. To extend our previous interest in looking for novel aminophenol derivatives of 5-FU with higher bioactivity and take advantage of the concept of bioisosterism, (S)-2-(5-Fluorouracil-1-Acetyl)-amido-1,4-succinic acid (5-FU-Asp), (S)-2-(5-Fluorouracil-1-Acetyl)-amido-3-Indolepropionic acid (5-FU-Trp), (S)-2-(5-Fluorouracil-1-Acetyl)-amido-3-hydroxypropionic Acid (5-FU-Ser), (S)-2-(5-Fluorouracil-1-Acetyl)-amido-3-(4-hydroxyphenyl)-propionate Acid (5-FU-Tyr), and (S)-2-(5-Fluorouracil-1-Acetyl)-amido-3-phenylpropionic acid (5-FU-Phe) that were derivatives of 5-FU, have been designed and synthesized and characterized (Fig. 1).



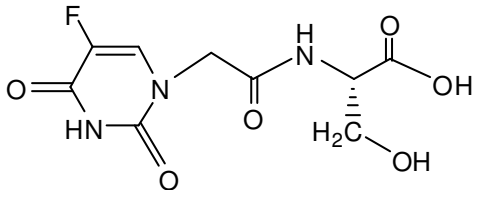
5-FU-Asp

A)



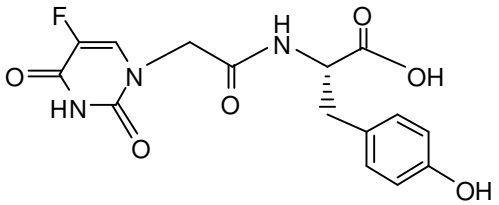
5-Fu- Trp

B)



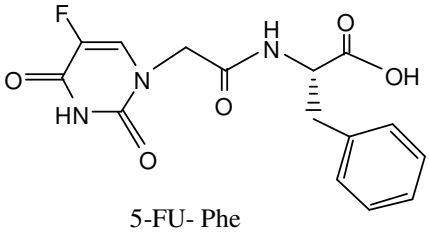
5-FU- Ser

C)



5-FU-L-Tyr

D)



5-FU- Phe

E)

Figure 1. The chemical structure of 5-Fluorouracil derivatives (A-E)

2. EXPERIMENTAL DESIGN

2.1. Chemicals and instrumentation

Electrochemical measurements were carried out with a model AUTOLAB PGSTAT30 electrochemical workstation (Metrohm AG) controlled by a personal computer. A conventional three-electrode system was used in the measurements at room temperature (25°C), with a bare or modified gold electrode (d = 2 mm) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt plate as the counter electrode. All the potentials given were referred to the SCE. Unless specially stated, the electrolyte solutions were thoroughly degassed with N_2 and kept under a N_2 blanket.

Calf thymus DNA (CT DNA obtained from Sino-American Biotechnical) was used as received. Solutions of DNA ($\approx 10^{-4}$ M in nucleotide phosphate NP) in 5.0 mM pH=7.20 Tris–HCl buffer solution containing 5.0 mM NaCl were purified to reach a high purity (A_{260}/A_{280} was larger than 1.8, where A represents the absorbance), indicating that the DNA could be used [27, 29, 30]. Stock solutions were stored at 4°C and used within three days. The concentration was determined by UV absorbency at 260 nm in the 1:100 diluted solutions. The extinction coefficient, ε_{260}, was taken as 6600 M^{-1} cm^{-1}. 5.0×10^{-3} M, and the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1M KCl was used as a redox probe in the electrochemical measurements.

Derivatives of 5-Fluorouracil and DNA were dissolved in 5.0 mM (pH=7.20) Tris–HCl buffer solution containing 5.0 mM NaCl which is used as the supporting electrolyte. Other chemicals were at least of analytical reagent grade. The buffer solution refers to 5.0 mM Tris–HCl buffer solution

containing 5mM NaCl supporting electrolyte. Ultra-pure water (18.22 MΩ cm^{-1}) was used for the preparation of all solutions.

2.2. Preparation of DNA-modified gold electrodes

The gold electrodes were first polished carefully with 1.0, 0.3 and 0.05μm alumina slurry and then cleaned ultrasonically in acetone, ethanol and water respectively, for 10 min. The real electrode area was estimated from cyclic voltammograms (CV) by integrating the cathodic peak for the reduction of the oxide layer in 0.5 M H_2SO_4. The freshly polished electrodes were scanned over the potential range of 0.0 to +1.5 V (vs. SCE) in 0.5 M H_2SO_4 until a constant voltammogram was obtained. Then, they were polarized at 0 V for 3 min. Finally, the electrodes were rinsed with water and modified immediately by transferring a droplet of 20 μL of 0.5 mM DNA solution onto the surface, followed by air-drying overnight. Then, the electrodes were soaked in sterile water for at least 4 h before being rinsed with water to remove any unadsorbed DNA. The DNA-modified gold electrodes thus obtained are denoted as DNA/Au in the text.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of DNA-modified electrode

Cyclic voltammetry of electroactive species $Fe(CN)_6^{3-/4-}$ has been used widely to test the kinetic of the interface barrier. The extent of kinetic hindrance to the electrontransfer process increased with increasing thickness and decreasing defect density of the barrier [31].

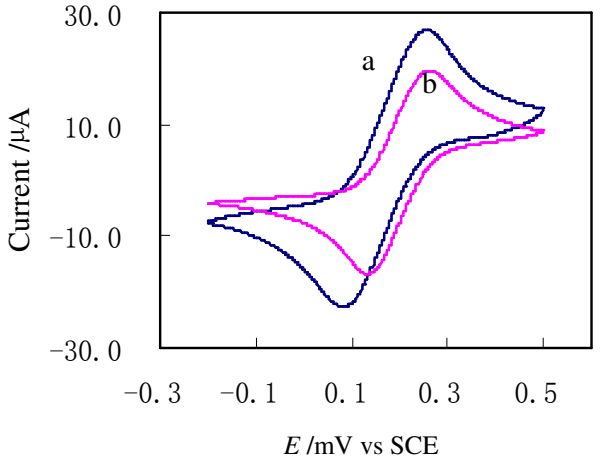


Figure 2. Cyclic voltammograms of 5 mM $Fe(CN)_6^{3-/4-}$ at a bare gold electrode (curve) and DNA/Au (curve b) respectively. Scan rate,100 mV/s.

Fig. 2 shows the cyclic voltammogram (CV) responses of 5 mM $Fe(CN)_6^{3-/4-}$ at bare Au and DNA/Au, respectively. $Fe(CN)_6^{3-/4-}$ produced a couple of well-defined redox waves at bare Au (Fig. 2, curve a) with a peak-to-peak separation (ΔE_p) of 94 mV at 100 mV/s. After the electrode was modified with DNA, an obvious decrease in the redox peak current was observed (Fig. 2, curve b), indicating that DNA acted as the inert electron and mass transfer blocking layer and thus hinders the diffusion of ferricyanide towards the electrode surface. This demonstrates that DNA has been successfully assembled on Au surface. The CV of the same DNA electrode remain stable after 20 scans in Tris-HCl buffer solution, suggesting the electrochemical stability of the DNA-coated film.

3.2. Interaction of dsDNA with 5-Fluorouracil derivatives

In order to investigate the interaction of DNA with 5-Fluorouracil derivatives, a dsDNA/Au electrode was put into buffer solution in two cases, i.e. presence and absence of 5-Fluorouracil derivatives, for voltammetric tests. The result was shown in Fig. 3. No peak was observed during the electrochemical scanning in the cases of both presence and absence of 5-Fluorouracil derivatives. The increases of charged and uncharged currents in the 5-Fluorouracil derivatives solution suggested that 5-Fluorouracil derivatives molecule probably interacts with DNA. In order to confirm the interaction, the DNA-modified electrode was scanned in buffer solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$ probe molecule, then 5-Fluorouracil derivatives was added into the test solution. The experiments showed the peak current of probe molecule decreased when 5-Fluorouracil derivatives was added into the test solution. The more 5-Fluorouracil derivatives were added, the more the peak current of probe molecule decreased. Therefore, the peak current decreased with respect to original peak current (Fig. 4, Table. 1), when the concentration of 5-FU-Asp, 5-Fu-Trp, 5-FU-Ser, 5-FU-Tyr, and 5-FU- Phe were adjusted to 4.73×10^{-4}M.

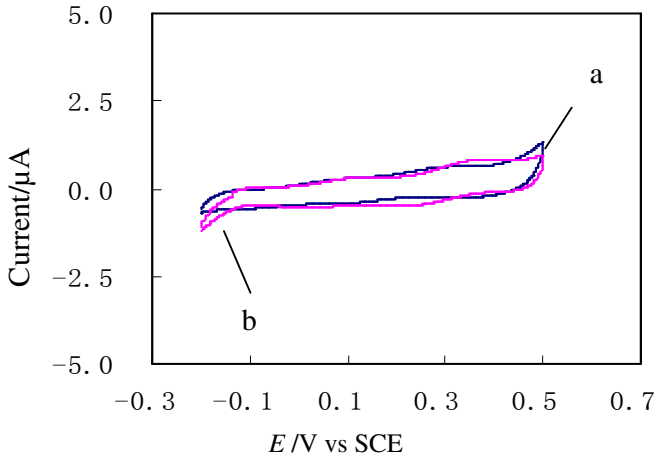


Figure 3. Cyclic voltammograms of dsDNA/Au electrode.a: dsDNA/Au electrode in 5.0mM pH=7.20 Tris–HCl buffer solution containing 5.0 mM NaCl without 5-Fluorouracil derivatives; b: dsDNA/Au electrode in the same buffer solution with 5.0×10^{-4} M 5-FU-Asp, 5-Fu-Trp, 5-FU-Ser, 5-FU-Tyr, and 5-FU- Phe; scan rate 100 mV/s.

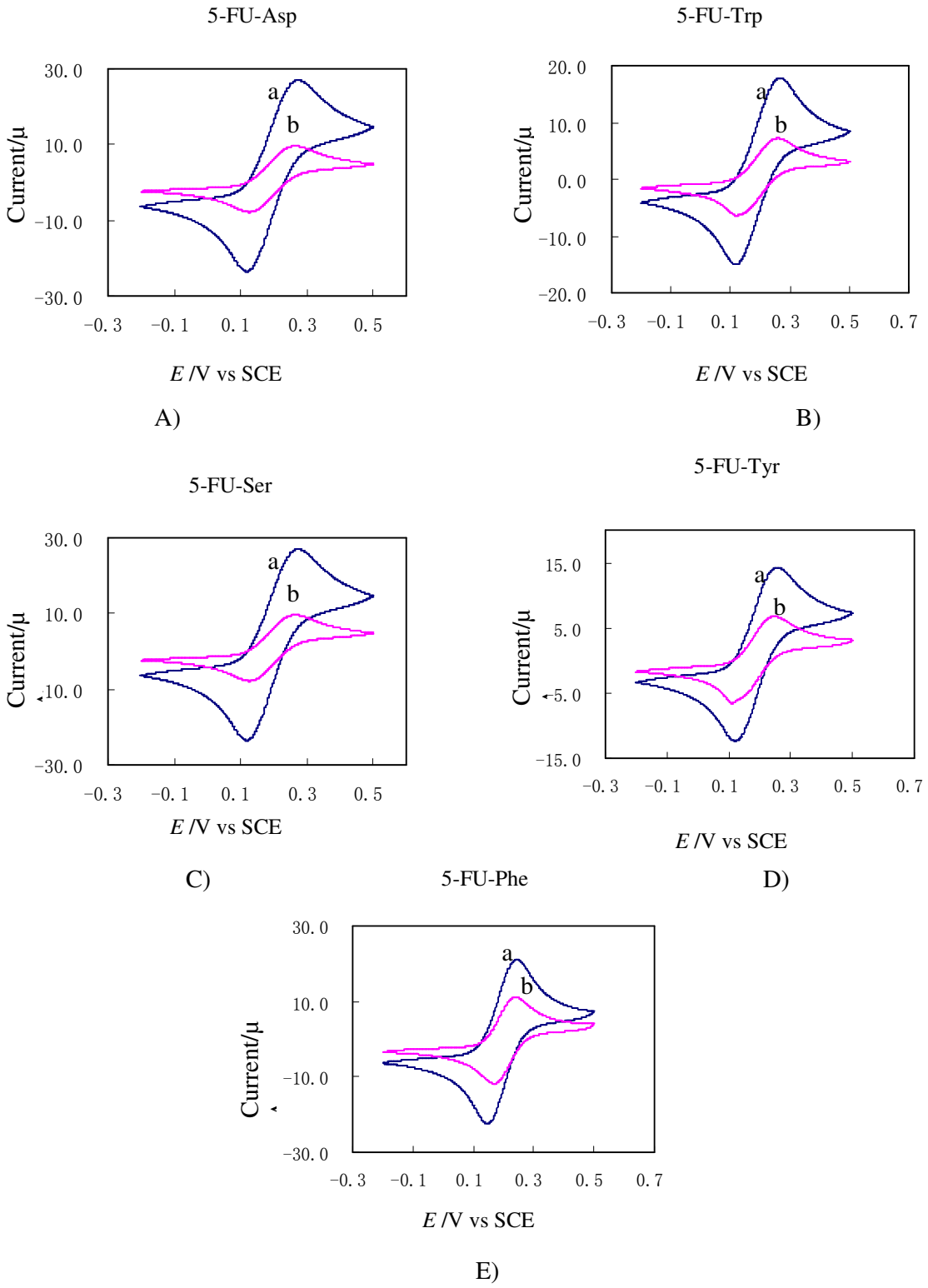


Figure 4. Cyclic voltammograms of a dsDNA/Au electrode in 5.0 mM $Fe(CN)_6^{3-/4-}$ pH=7.20 solution containing 5.0 mM NaCl supporting electrolyte (A-E) without five 5-Fluorouracil derivatives (curve a) and with 4.73×10^{-4}M five 5-Fluorouracil derivatives (curve b). scan rate 100 mV/s.

The maximum respective peak current changes were 1.82×10^{-6} A, 1.03×10^{-6} A, 1.08×10^{-6} A, 8.10×10^{-7} A, and 1.18×10^{-6} A, respectively. The phenomenon confirmed that 5-Fluorouracil derivative molecules interacted with dsDNA. Assuming the 5-Fluorouracil derivatives were not protonated in the common neutral buffer solution (pH 7.20), the electrostatic interaction between DNA and 5-Fluorouracil derivatives could be ignored. When the probe molecules of $Fe(CN)_6^{3-/4-}$ got in double-strand of DNA [32], the 5-Fluorouracil derivative molecules were added to the test solution and competed for sites at DNA with probe molecule that led to a decrease in the peak current of the probe molecules. So, it could be speculated that 5-Fluorouracil derivatives targeted in the double-stranded DNA. On the other hand, the addition of DNA made the peak potential shifted in a positive direction, confirming the dominance of intercalative interaction between rutin and DNA.

Table 1. Determination of binding constants

Drug	$\Delta I_{max}/A$	β	m
5-FU-Asp	1.82×10^{-6}	3.23×10^{12}	3.5
5-Fu-Trp	1.03×10^{-6}	5.6×10^{7}	2
5-FU-Ser	1.08×10^{-6}	3.23×10^{8}	2
5-FU-Tyr	8.10×10^{-7}	2.10×10^{10}	3
5-FU-Phe	1.18×10^{-6}	5.19×10^{9}	2.7

3.3. Determination of association constant and binding number between Drug and DNA

According to the method of Qu *et al*. [33] and Shen *et al*. [34], it is assumed that DNA and DRUG only produce a single complex DNA· $DRUG_m$. The stoichiometric coefficient, *m*, and association constant, *β*, between Drug and DNA refer to the reaction scheme (1) for allor-none (Hill) cooperativity of multiple ligand binding:

$$DNA + m\ DRUG \quad DNA\cdot DRUG_m \qquad (1)$$

The condition of association constant is as follows:

$$\beta\cdot [DRUG]_m = [\ DNA\cdot DRUG_m\ ]/[\ DNA\ ] = f/1-f \qquad (1.1)$$

where $f = [DNA\cdot DRUG_m] / [DNA]_0$ is the fraction of DNA to relative to the total DNA concentration in the supporting electrolyte $[DNA]_0 = [DNA\cdot DRUG_m]_{max}$.

Mass conservation dictates that: $[DNA] = [DNA]_0 - [DNA.\ DRUG_m]$, then,

$$[\text{Drug}] = [\text{DRUG}]_0 - m[\text{DNA. DRUG}_m] \quad (1.2)$$

and

$$I = k\cdot [\text{DRUG}] \quad (1.3)$$

$$\Delta I = I(\text{DRUG})_0 - I(\text{DRUG}) \quad (1.4)$$

where [DRUG] is the free concentration of DRUG and I(DRUG) is the peak current of DRUG in the presence of DNA.

Insertion of Eqs. (1.2) and (1.3) into (1.4) yields:

$$\Delta I = k([\text{DRUG}]_0 - [\text{DRUG}]) = k\cdot m\cdot [\text{DNA. DRUG}_m] \quad (1.5)$$

and

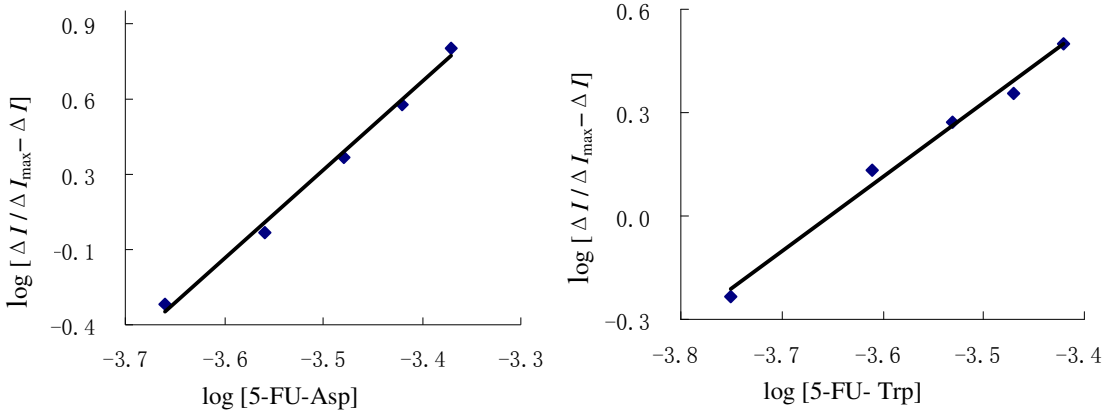
$$\Delta I_{\max} = k\cdot m\cdot [\text{DNA}]_0 \quad (1.6)$$

where ΔImax is the maximum peak current change, obviously, $[\text{DNA. DRUG}_m]_{\max}=[\text{DNA}]_0$ holds true. Based on the equations above, the followings can be deduced:

$$\log(\Delta I / \Delta I_{\max} - \Delta I) = \log\beta + m \log [DRUG] /\text{mol/L} \quad (1.7)$$

$$1/\Delta I = 1/\Delta I_{\max} + 1/\beta\cdot \Delta I_{\max}).1 / [\text{DRUG}]_m) \quad (1.8)$$

The corresponding experimental data are shown in Fig. 5. The log $[\Delta I / \Delta I_{\max} - \Delta I]$ vs. log {[DRUG]/(mol/L)} becomes linear with the slope of m. The results of m and β are shown in table 1.from which we can see that β (DNA. 5-FU-Asp)> β (DNA. 5-FU-Tyr)> β (DNA. 5-FU-Phe)> β (DNA. 5-FU-Ser) β (DNA. 5-FU-Trp).



A) B)

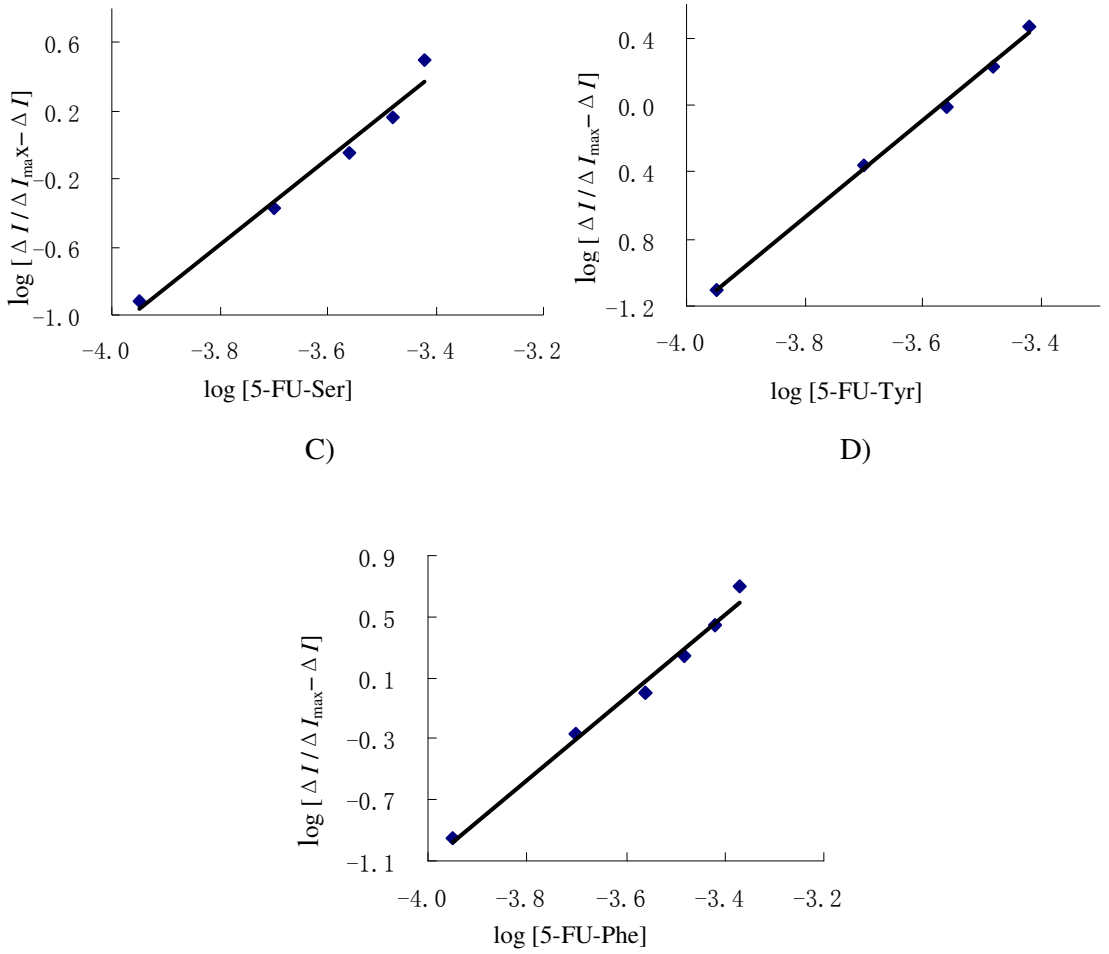


Figure 5. The relationship between log[$\Delta I/(\Delta I_{max} - \Delta I)$] and log [derivatives of 5-Fluorouracil] at the DNA/Au in the 5 mM/L Tris–HCl (pH 7.2) buffer solution (A-E)

4. CONCLUSIONS

In this work, the electrochemical behavior of 5-Fluorouracil derivatives and their interaction with DNA were investigated by electrochemical methods. All the experimental results indicate that the principal interaction mode of 5-Fluorouracil derivatives with DNA is cooperative interaction. The interaction can be quantified in terms of the Hill model for cooperative interactions. The results demonstrate that the electrochemistry is available and significantly promising for further studies on the mechanism of DNA interaction with targeting compounds. The results can also serve as a reference for synthesis, structural characterization of new 5-Fluorouracil derivatives.

Acknowledgments: We are grateful to the reviewers and editor for their constructive comments and suggestions. This work was supported by Chinese grants of Anhui Educational Research Funds to Dr. Liu, Wu-yi (2005QL11, 2006jql222, 2006KJ224B).

References

1. Y.Q. Li, Y.J. Guo, X.F. Li, J.H. Pan, *Talanta*. 71 (2007) 123.
2. H. Li, W. Mei, Z. Xu, D. Pang, L. Ji, Z. Lin, *J. Electroanal. Chem.* 600 (2007) 243.
3. C. Li, S. Liu, L. Guo, D. Chen, *Electrochem. Commun.* 7 (2005) 23.
4. K.D. Sugden, K.M. Rigby, B.D. Martin, *Toxicol. In Vitro*. 18 (2004) 741.
5. A.Chiou, R. Verger, K. G., *Lipids*. 36 (2001) 535.
6. A.Chiou, R. Verger, G. Kokotos, *Lipids*. 36 (2001) 535.
7. G. Octobre, C. Lemercier, S. Khochbin, M. Robert-Nicoud, C. Souchier, *Cr Biol.* 328 (2005) 1033.
8. M. Eisenstein, Z. Shakked, *J. Mol. Biol.* 248 (1995) 662.
9. A.Szilagyi, G.K. Bonn, A. Guttman, *J. Chromatogr. A*. 1161 (2007) 15.
10. H. Heli, S.Z. Bathaie, M.F. Mousavi, *Electrochem. Commun.* 6 (2004) 1114.
11. J.M. Séquaris, J. Swiatek, *Bioelectrochemistry and Bioenergetics*. 26 (1991) 15.
12. P. Yin, M. Hu, L. Hu, *J. Mol. Struct.* 882 (2008) 75.
13. X.Wang, J. Lin, X. Zhang, Q. Liu, Q. Xu, R. Tan, Z. Guo, *J. Inorg. Biochem.* 94 (2003) 186.
14. A.T. Hulme, S.L. Price, D.A. Tocher, *J. Am. Chem. Soc.* 127 (2005) 1116.
15. S. Akyuz, T. Akyuz, Y. Akkaya, E. Akalin, *J. Mol. Struct.* 834-836 (2007) 403.
16. M. Nichifor, E.H. Schacht, L.W. Seymour, *J. Control. Release*. 48 (1997) 165.
17. W.D. Wang, M.L. Hu, *Chinese J. Struc. Chem.* 25 (2006) 562.
18. T.B. Cai, X. Tang, J. Nagorski, P.G. Brauschweiger, P.G. Wang, *Bioorgan. Med. Chem.* 11 (2003) 4971.
19. M. Maeda, N. Kajimoto, Z. Yamaizumi, Y. Okamoto, K. Nagahara, H. Takayanagi, *Tetrahedron Lett.* 38 (1997) 6841.
20. S. Akyuz, T. Akyuz, *J. Mol. Struct.* 924-926 (2009) 37.
21. R. Kannappan, D.M. Tooke, A.L. Spek, J. Reedijk, *Inorg. Chim. Acta*. 359 (2006) 334.
22. R. Kannappan, S. Tanase, D.M. Tooke, A.L. Spek, I. Mutikainen, U. Turpeinen, J. Reedijk, *Polyhedron*. 23 (2004) 2285.
23. J. Xiong, X.X. Lei, M.L. Hu, J.X. Yuan, X.Q. Cai, *Zeitschrift fur Kristallographie - New Crystal Structures*. 221 (2006) 37.
24. K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Deliver. Rev.* 47 (2001) 113.
25. R. Kannappan, D.M. Tooke, A.L. Spek, J. Reedijk, *J. Mol. Struct.* 751 (2005) 55.
26. R. Kannappan, D.M. Tooke, A.L. Spek, J. Reedijk, *J. Mol. Struct.* 751 (2005) 55.
27. X. Li, Y. Chen, X. Huang, *J. Inorg. Biochem.* 101 (2007) 918.
28. B.Meric, K. Kerman, D. Ozkan, P. Kara, S. Erensoy, U.S. Akarca, M. Mascini, M. Ozsoz, *Talanta*. 56 (2002) 837.
29. E.B. Feng (Ed.), Proceedings of the First China Postdoctoral Academic Congress, National Defense Industry Press, Beijing, 1993.
30. A.J.S. Ahammad, S. Sarker, M.A. Rahman, J. Lee, *Electroanal.* 22 (2010) 694.
31. L. Wang, J. Bai, P. Huang, H. Wang, L. Zhang, Y. Zhao, *Electrochem. Commun.* 8 (2006) 1035.
32. V. Ramakrishnan, M. D'Costa, K.N. Ganesh, M. Sastry, *J. Colloid Interf. Sci.* 276 (2004) 77.
33. F. Qu, N. Li, Y. Jiang, *Talanta*. 45 (1998) 787.
34. H. Shen, H. Zheng, N. Zhu, Y. Liu, J. Gao, J. Li, *Int. J. Electrochem. Sci.*, 5(2010)1587.