Electrochemical and Spectroscopic Studies on the Interaction Between DNA and the Chromotrope 2R

S. NIU, L. HU, S. ZHANG*
College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, 266042 Qingdao, Shandong, P. R. China.

(Received 13th August, 2004, revised 3rd September, 2005)

Summary: The electrochemical behavior of chromotrope 2R (CT2R) on a glass carbon electrode was studied in 0.2 mol·L^{-1} Britton-Robinson (B-R) buffer solution (pH 5.10). The interaction between CT2R and deoxyribonucleic acid (DNA) was investigated by cyclic voltammetry, differential pulse voltammetric curve and UV/ Vis spectroscopy. It was found that the oxidative peak current decreases significantly with a negative shift of the oxidative peak potential in the presence of DNA compared with that in the absence of DNA. The effect of natural DNA and denatured DNA on the CT2R system was studied, which indicated that the major binding mode of CT2R to DNA is "electrostatic binding". The binding ratio of CT2R-DNA complex is calculated to be 2: 1 and the binding constant is 5.68×10^5 mol^{-2}·L^2 at room temperature.

Introduction

Deoxyribonucleic acid (DNA) is the most important germ-plasm of most organisms. It plays an important role in the process of storing, copying and transmitting germ messages. Gradually, the research on the interaction between small molecules and DNA has become a field of increasingly general interest [1].

The spectroscopic and electrochemical methods are of potential importance to probing the interaction of small molecules with DNA and the detection of DNA. These researches have contributed to the understanding of the way of interaction between DNA and protein. Moreover, these researches are helpful to expound the action mechanism of anticancer drugs, the external selection of drugs and carcinogenesis of the carcinogenic compounds [2].

Azoic compound is one of the synthetic dyes, which are currently age-old and have maximal dosage. Now, more than three thousand azoic dyes are being used in the world [3], involving spin, papermaking, plastics, medical food etc, most of which are monozao-dyes. Many azoic compounds have been proved to be carcinogenic [4-5], whose degradable and carcinogenic mechanism is paving the way for broad attention and investigations. As reported [3, 6-10], anaerobes can deoxidize certain azoic compounds in the absence of oxygen. The anaerobes' frame is related to the degradation and degradable velocity. Therefore, the study of the reaction mechanism and kinetics can provide useful strategies to be adopted for bio-degradation and carcinogenic mechanism. In this article, the interaction of natural DNA (dsDNA) and denatured DNA (ssDNA) to the CT2R system was studied with electrochemical methods and UV/Vis spectroscopy. The conclusion obtained was that they possess electrostatic binding between CT2R and DNA. The binding ratio and the binding constant of the CT2R-DNA complex were also deduced.

Results and Discussion

Studies of Reaction Conditions Between CT2R and DNA

The Electrochemical Studies of CT2R on the Glass Carbon Electrode

Electrochemical activities were detected in the base solution of 0.1 mol·L^{-1} NaH_2PO_4-Na_2HPO_4, 0.2 mol·L^{-1} B-R, 0.05 mol·L^{-1} Tris-HCl or 0.1 mol·L^{-1} HOAc-NaOAc buffer solution. 0.2 mol·L^{-1} B-R buffer solution was selected as the base solution with the peaks being the best, which are shown in Fig. 1.

A quasireversible redox wave and an irreversible wave are shown in Fig. 1. The separation of oxidation and reduction peak potentials, ΔE_p, is 57

*To whom all correspondence should be addressed.

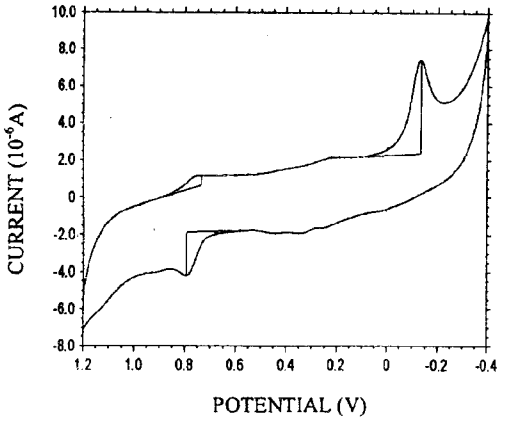

Fig. 1: The cyclic voltammetric curve of CT2R C_{CT2R}: 6.02×10^{-5} mol·L^{-1}.

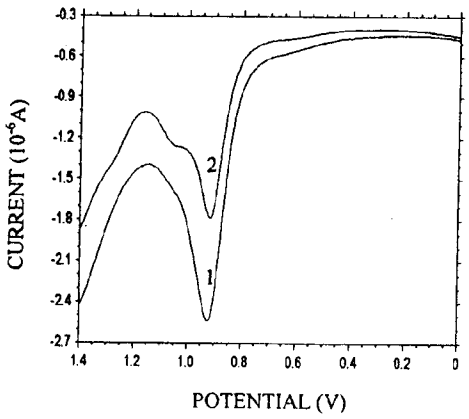

Fig. 2: The differential pulse voltammetric curve of CT2R C_{CT2R}: 6.02×10^{-5} mol·L^{-1} C_{DNA}: (1) 0; (2) 1.87×10^{-4} mol·L^{-1}.

mV. According to Nicholson's Kinetics of Electrode Process of the single-sweep voltammetry [11], it is a quasireversible two-electron transfer reaction. Under the selected conditions, the influence of pH to the peak potential of CT2R was studied. The potential of the oxidation peak shifts negatively with the augment of pH, which indicates that H^+ participates in the electrode reaction. The reduction peak potential has a good linear relation with the pH value in the range of 2.8~3.4 and the slope is –0.0584. According to the formula [12]: -0.059 x/n= -0.0584, where n is the number of the electron transfer, x is the hydrogen ion number participating in the reaction and x=n. From the experimental results, the redox processes of CT2R may be expressed as follows.

The relationship between I_{pa} and the concentration of CT2R shows that I_{pa} is directly proportional to the concentration of CT2R with a regression equation: y = 0.4405 x + 0.0986 and a correlation coefficient γ= 0.9977, where y is the voltammetric oxidation peak current I_{pa} and x is the concentration of CT2R.

The curve 2 of Fig. 2 is the DPV curve of 6.02×10^{-5} mol·L^{-1} CT2R in the presence of 1.87×10^{-4} mol·L^{-1} DNA compared with the curve 1 in the absence of DNA. The results show an obvious decrease of the peak current with a negative shift of the oxidative peak potential after adding DNA. No new oxidation-reduction peaks however appeared. So CT2R interacting with DNA forms electrochemically non-active complex [13]. In the presence of DNA, the equilibrium concentration of CT2R decreases, which results in a decrease of the peak current. It is generally accepted that there are three kinds of binding modes for small molecules to DNA, which refer to intercalative binding, groove binding and electrostatic binding. Bard [14] has reported that if the oxidative peak potential shifts to a more negative value when small molecules interact with DNA, the interaction mode is electrostatic binding. On the contrary, if the oxidative peak potential shifts to more positive value, the interaction mode is intercalative binding. According to the molecular structure of CT2R and this negative shift of peak potential, the initial conclusion drawn could be that the major interaction mode of CT2R with DNA is "electrostatic binding".

The Influence of the pH on the Action

Fig. 3 shows the relationship curve between the variation of the oxidation peak current of CT2R before and after adding DNA. Initially, the oxidation peak current of CT2R increased, and reached a maximum at a pH of 5.10. Soon after, it immediately decreased quickly. Consequently, 5.10 was chosen as the best pH of the reaction.

The Influence of the Scan Rate on the Peak Current of CT2R

I_{pa} is nonlinear to $\nu^{1/2}$, which is shown in Fig. 4. This indicates that the electrode process of CT2R is not only controlled by the diffusion of CT2R, but also by the surface action. There is enrichment on the surface of the glass carbon electrode [15], which shows the character of surface absorption of CT2R.

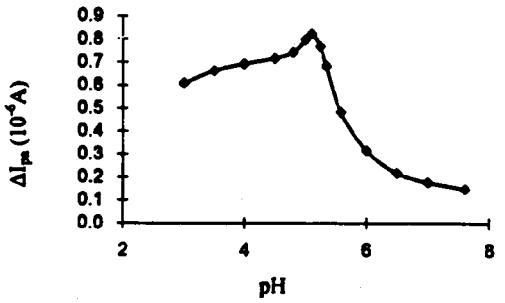

Fig. 3: The relationship curve of ΔI_{pa} and pH C_{CT2R}: 6.02×10^{-5} mol·L^{-1} C_{DNA}: 2.81×10^{-4} mol·L^{-1}

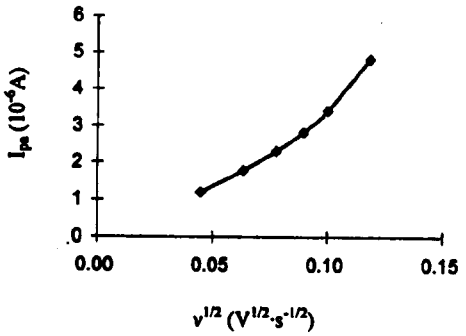

Fig. 4: The relationship curve of I_{pa} and $v^{1/2}$ C_{CT2R}: 6.02×10^{-5} mol·L^{-1}.

The Influence of the Reacting Time on the Peak Current of CT2R

The relationship between the oxidation peak current and the reaction time was studied after CT2R and DNA were mixed under room temperature Fig. 5.

The peak current of CT2R gradually decreased with the increasing reaction time and finally reaching a constant value. The variation of the oxidation peak current reached the maximum at the 5th min. About 10 min later, the peak current almost did not change, indicating that the reaction of CT2R with DNA had reached an equilibrium state. Therefore, 10 min was deduced as the reaction time.

The Binding Ratio and the Binding Constant of the CT2R-DNA Complex

According to the reference [16], it is assumed that there is only one complex of DNA-n CT2R.

DNA+nCT2R ═══ DNA-nCT2R (n=1,2,3, or 1,1/2,1/3)

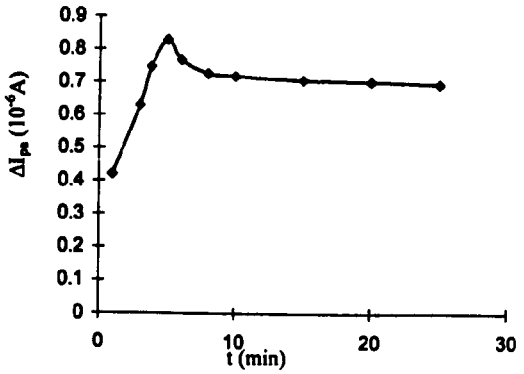

Fig. 5: The influence of the reaction time on the peak current of CT2R and DNA C_{CT2R}: 6.02×10^{-5} mol·L^{-1} C_{DNA}: 2.81×10^{-4} mol·L^{-1}.

The equilibrium constant can be expressed as follow,

$$\beta = \frac{[DNA - nCT2R]}{[DNA][CT2R]^n} \quad (1)$$

And the following equations can be deduced,

$$\Delta Imax = K'C_{DNA} \quad (2)$$

$$\Delta I = K'[DNA\text{-}nCT2R] \quad (3)$$

$$[DNA]+[DNA\text{-}nCT2R]=C_{DNA} \quad (4)$$

$$\Delta Imax-\Delta I = K'(C_{DNA}-[DNA\text{-}nCT2R]) \quad (5)$$

$$\Delta Imax-\Delta I = K'[DNA] \quad (6)$$

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{\beta \Delta I_{max}[CT2R]^n} \quad (7)$$

With different n, there are different relationship curves of ΔI^{-1} and $[CT2R]^{-n}$. According to equation (7), the relationship curve of ΔI^{-1} and $[CT2R]^{-n}$ should be a straight line with the suitable n if there is only one complex formed. From the slope and intercept of the best line, the binding constant β of the complex of DNA-nCT2R can be calculated.

In Fig. 6, curve 1 shows the relationship of I_{pa} and C_{CT2R} in the absence of DNA. Curve 2 typically represents the current change at C_{DNA} = 2.81×10^{-4} mol·L^{-1} on varying the concentration of CT2R. Curve

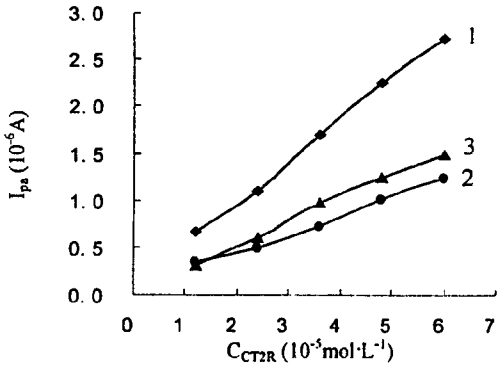

Fig. 6: The relationship curve of I_{pa} and ΔI_{pa} vs. C_{CT2R} (1) C_{DNA}: 0; (2) C_{DNA}: 2.81×10^{-4} mol·L^{-1}; (3) $\Delta I_{pa}=I_{pa1}-I_{pa2}$.

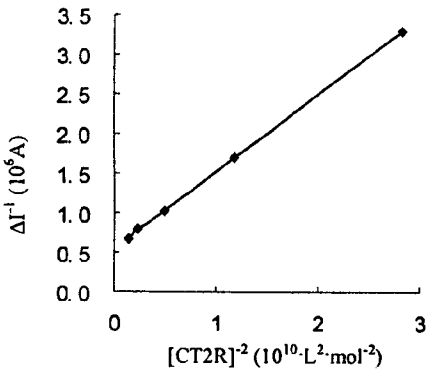

Fig. 7: The relationship curve of ΔI^{-1} vs. $[CT2R]^{-2}$.

3 is the relationship between ΔI, which means the $(I_{pa1}-I_{pa2})$ value and the concentration of CT2R. As for n=2, the relationship curve of ΔI^{-1} and $[CT2R]^{-n}$ is a straight line (γ=0.9998), as shown in Fig 7. While for n=1/2 and 1, the curve bends up. From the slope and intercept of the best line between ΔI^{-1} and $[CT2R]^{-2}$, the binding constant β calculated to be 5.68×10^5 L^2·mol^{-2}, which corresponded to the following equation: DNA + 2CT2R $\rightleftharpoons$ DNA-2 CT2R

The Effect of the Concentration of DNA on the Oxidation Peak Current of CT2R

Fig. 8 shows the relationship between the oxidation peak current of CT2R and DNA concentration, while the concentrations of both dsDNA and ssDNA increased gradually. At the beginning, the peak current decreased. When the concentration of DNA increased to a certain degree,

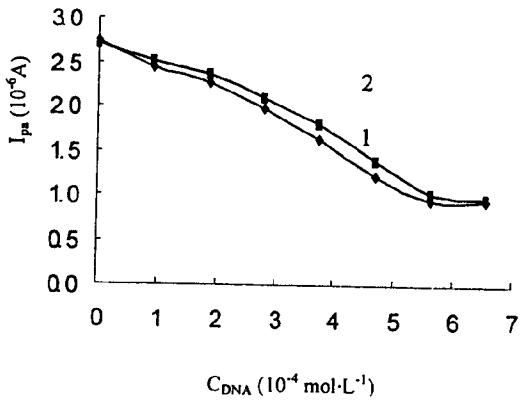

Fig. 8: The influence of the concentration of ds DNA (curve 1) and ss DNA (curve 2) on the peak current of CT2R C_{CT2R}: 6.02×10^{-5} mol·L^{-1}

the peak current reached a constant value. Eventually, the peak current decreased no longer, suggesting that the interaction of CT2R with DNA to be saturated.

According to the literature [17], for the intercalative binding in which the intercalator can provide a planar heterocyclic molecular surface for efficient intercalation into dsDNA strand, the reduction effect of dsDNA concentration on the peak current is very obvious, while ssDNA has almost no reduction effect on it. According to Fig. 8, both dsDNA and ssDNA concentrations had reduction effect on the peak current of CT2R. There was no obvious difference between them, indicating that the binding mode of CT2R to DNA was not through intercalative binding, but "electrostatic binding".

With reference to the method of John Wiley & Sons [18], the formula for measuring the peak current is as follows: I_{pa}= 269 $n^{3/2}$ A $D^{1/2}v^{1/2}$ C_0. The relationship between the peak current (I_{pa}) and the diffusion coefficient (D) can be deduced. After adding DNA, the diffusion coefficient of the association complex (CT2R-DNA) becomes much smaller than that of the free complex (CT2R) when CT2R binds to DNA, which results in the reduction of the diffusion velocity and the decrease of the peak current.

The UV/V is Spectroscopic Studies of the Interaction Between CT2R and DNA

The interaction between CT2R and DNA was studied. The variations of CT2R spectra in the

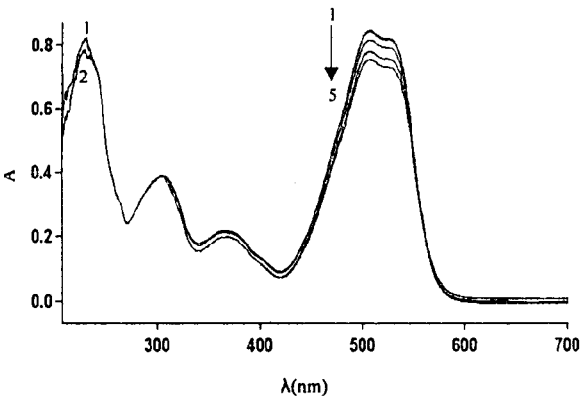

Fig. 9: The UV/Vis spectra of CT2R with different concentration of natural DNA C_{CT2R}: 4.01×10^{-5} mol·L^{-1}; C_{DNA}: (1) 0; (2) 6.32×10^{-5} mol·L^{-1}; (3) 3.12×10^{-4} mol·L^{-1}; (4) 7.80×10^{-4}mol·L^{-1}; (5) 1.56×10^{-3}mol·L^{-1}

presence of different concentrations of dsDNA are shown in Fig. 9. It was observed that all the absorption peaks of CT2R at 234.9 nm, 306.0 nm, 365.9 nm, 508.9 nm decreased with the increasing DNA concentration, especially at 234.9 nm and 508.9 nm. The peaks at 234.9 nm and 508.9 nm shifted to 231.0 nm and 507.0 nm, which indicated that CT2R can interact with DNA via electrostatic interaction. The result was consistent with the above electrochemical studies.

No obvious differences were found in the absorption spectra after the same concentrations of ssDNA were added to the above CT2R solution. Only nuance was observed in the ultraviolet, indicating that there was little intercalation between CT2R and dsDNA. In addition, the binding interactions of EB with DNA were examined in the presence of CT2R solution, in the hope of providing information about the similarities or differences in the nature of the binding modes of these complexes to DNA. The absorption spectroscopy of EB showed that EB has an absorption peak at 478 nm in the buffer solution. There are phenomena of hypochromic effect and red shift in the presence of DNA. These phenomena result from the intercalation between EB and DNA. It is generally recognized that the strong mode of binding of EB to dsDNA results in the intercalation of the planar phenanthridinium ring between adjacent base pairs on the double helix [19-20]. After the addition of CT2R, the absorption peak of EB does not increase, which has proven that the interaction between CT2R and DNA is different from the intercalation between EB and DNA. Based on the above experimental results, the conclusion can be drawn that CT2R interacts with DNA via electrostatic interaction.

Experimental

Apparatus and Reagents

CHI832 electrochemical analyzer, produced by Shanghai Chenhua Instrument Company of China; the three-electrode system composed of a glass carbon electrode (GCE) as working electrode, a Ag/AgCl as the reference electrode and a platinum electrode as auxiliary electrode; Cary50 UV/Vis spectrophotometer, produced by Varian Company of Australia; pHS-25 pH meter, produced by Shanghai Leici Instrument Factory of China.

Chromotrope 2R (CT2R) was purchased from Shanghai Chemical Reagent Company of China; Salmon sperm DNA was purchased from Huashun Biologic Engineering Company of Shanghai. Its concentration was calculated by the absorbance of 260 nm (ε = 6600 L·mol^{-1}·cm^{-1}). Britton-Robinson (B-R) buffer solution, with a pH was adjusted to 5.10 was also med the other reagents were all analytical reagents prepared with doubly deionized water.

Procedure

Electrochemical Studies of the Interaction Between CT2R and DNA

50 μL of 6.02×10^{-3} mol·L^{-1} CT2R was added to 5 mL of pH 5.10 B-R buffer solution, to which the different quantities of salmon sperm DNA were added. The cyclic voltammetric (CV) curve and differential pulse voltammetric (DPV) curve figures were recorded on the CHI832 electrochemical analyzer.

UV/Vis Spectroscopic Studies of the Interaction Between CT2R and DNA:

20 μL of 6.02×10^{-3} mol·L^{-1} CT2R and different volume of 4.68×10^{-2} mol·L^{-1} dsDNA and ssDNA solution were in turn added to 10 mL colorimetric tubes respectively, then diluted to the desired scale with B-R buffer solution. The solutions were set for 10 min at room temperature. The UV/Vis spectra were recorded on a Cary 50 spectrophotometer in 1 cm quartz cuvettes. The range of the scanning wavelengths was from 200 nm to 800 nm.

Conclusions

The interaction between CT2R and DNA was investigated by cyclic voltammetry, differential pulse voltammetric curve and UV/Vis spectroscopy. It was found that the oxidation peak current decreases significantly with a negative shift of the oxidation peak potential in the presence of DNA as compared with that in the absence of DNA. The effect of natural DNA and denatured DNA on the CT2R system was studied, which indicated that CT2R can bind to DNA to form a 2:1 association complex with the binding constant of 5.68×10^5 $mol^{-2}\cdot L^2$ and the major binding mode of CT2R to DNA is "electrostatic binding". At room temperature, the electrochemical behavior of CT2R on a glass carbon electrode was studied in 0.2 $mol\cdot L^{-1}$ B-R buffer solution (pH 5.10) with the full reaction time being about 10 min.

Acknowledgements

The work was supported by the Natural Science Foundation of Shandong Province (Z2006B01, Y2006B07), the Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (No. O4B0021400), and the Outstanding Adult-young Scientific Research Encouraging Foundation of Shandong Province (No. 2005BS04007).

References

1. R. Y. Zhang, D.W. Pang, and R. X. Cai, *Chem. J. Chinese Universities,* **20** (8), 1210 (1999).
2. H. Z. Huang, and X. R. Yang, *Chinese J. Anal. Chem.,* **30** (4), 491 (2002).
3. K. T. Chung, and S. E. Stevens, *Crit. Rev. Microbiol.*, **18** (3), 175 (1992).
4. J. T. Spadaro, L. Isabelle, and V. Renganathan, *Environ. Sci. Technol.*, **28** (7), 1389 (1994).
5. W. X. Yang, *Environ. Chem.*, **2** (2), 1 (1983).
6. K. T. Chung, and S. E. Stevens, *Environ. Toxicol. Chem.*, **12**, 2121 (1993).
7. K. T. Chung, G. E. Fulk and M. Egan, *Appl. Environ. Microbiol.*, **35** (3), 558 (1978).
8. F. Rafii, W. Franklin and C.E. Cerniglia, *Appl. Environ. Microbiol.*, **56**, 2146 (1990).
9. J. J. Roxon, A. J. Ryan, and S. E. Wright, *Fd. Cosmet. Toxicol.*, **5** (3), 367 (1967).
10. Y. Urushigawa, and Y. Yonezawa, *Bull. Eviron. Contam. Toxicol.*, **17**, 214 (1977).
11. R.S. Nicholson, *Anal. Chem.*, **37** (11), 1351 (1965).
12. J. Everse, K. E. Everse, and M. B. Crisham, Peroxidase in Chemistry and Biology, Vol II, Boston, CRC Press. 5 (1991).
13. D. J. Liu, Z. Z. Wang, and S. Dong, *Chinese Journal of Analysis Laboratory,* **21** (4), 54 (2002).
14. M. T. Carter, M. Rodriguez, and A. J. Bard, *J. Am. Chem. Soc.*, **111** (24), 8901 (1989).
15. K. Yamasaki, T. Hara and M. Yasuda, *Proc. Jpn. Acd.* **29**, 337 (1953).
16. N. Li and J. Min, *Chinese J. Anal. Chem.*, **17** (4), 346 (1989).
17. J. Liu, J. Li and S. Dong, *Electroanal*, **8**, 803 (1996).
18. Jhon Wiley & Sons, *Electrochemical methods: Fundamendal and Applications.* New York, pp. 218 (1980).
19. G. R. Christian and R. K Thomas, *Biochem.*, **14**, 4845 (1978).
20. J. X. Lu, G. Z. Zhang, Z. N. Huang, and P. Zhao, *Acta Chimica Sinica*, **60**, 967 (2002).