Chinese Journal of Chemistry, **2004**, *22*, 1325—1329 **Article**

Studies on the Interaction between Rutin and DNA in the Absence and Presence of β-Cyclodextrin by Electrochemical and Spectroscopic Methods

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The interactions between rutin or the inclusion complex of rutin-β-cyclodextrin and DNA were investigated by means of cyclic voltammetry, UV-vis absorption spectroscopy and fluorescence emission spectroscopy. The apparent binding constant of rutin with DNA is found to be 2.9×10^4 L/mol. The results showed that the benzopyranic-4-one plane of rutin mainly intercalated into DNA in the absence of β-cyclodextrin, while the catecholic portion of rutin was located in the double helix of DNA in the presence of β-cyclodextrin.

Keywords rutin, DNA, β-cyclodextrin, cyclic voltammetry, UV-vis absorption spectroscopy, fluorescence emission spectroscopy, interaction

Introduction

The interaction between various molecules and DNA is an important fundamental issue in life science that is related to the replication and transcription of DNA *in vivo*, mutation of genes and related variations of species in character, action mechanisms of some DNA-targeted drugs, origins of some disease, and action mechanisms of some synthetic chemical nucleases and so on. Numerous methods, including UV-vis spectroscopy,[1,2] fluorescence spectroscopy,[2] Raman spectroscopy[3] and X-ray,[4] have been used to study these interactions.

Electrochemical methods have also been used to study the interactions of other molecules with DNA in solution. Some metal chelates, such as Co(III),[5,6] Ru(II)[7] and Os(II),[8] with 1,10-phenanthroline and 2,2"-bipyridyl bound to DNA have been studied by cyclic voltammetry, differential pulse voltammetry, chronocoulometry, and electrogenerated chemiluminescence. Recently, surface electrochemical methods, namely the use of DNA-modified electrodes, have been used for investigating the interaction between other molecules and DNA.[9] Electrochemical investigation of the interactions of nucleic-acid-binding molecules and DNA[10,11] can provide a useful complement to the previously used methods, such as spectroscopic methods.

Rutin is one of the bioactive flavonoid compounds, which are present in substantial amounts in plants. Some related investigations showed that rutin has a broad range of physiological activities. Bao[12] *et al.* pointed out that an electrochemical inactive supromolecular complex of rutin-DNA was formed by cyclic voltammetry. Solimani[13] indirectly suggested that probably only the benzopyranic-4-one portion of rutin, as well as quercetin, could be located into the biopolymer, whereas the coplanar catecholic portion would more likely be oriented towards the external aqueous medium by linear dichroism technique. As well known, β-cyclodextrin (β-CD) can provide a hydrophobic cavity in aqueous solution for the hydrophobic molecules or groups to form inclusion complexes. The 1∶1 inclusion complex of rutin with β-CD (denoted as rutin-β-CD) was studied by thin-layer chromatography,[14] reversed-phase liquid chromatography[15] and fluorimetry.[15,16]

Recently, the interaction modes between small molecules and DNA are investigated using cyclic voltammetry and spectroscopic methods by means of cyclodextrin.[17-19] In order to reveal the interaction site between rutin and DNA, the interaction mechanism of rutin and the inclusion complex rutin-β-CD with DNA has been respectively investigated by cyclic voltammetric and spectroscopic methods. The experimental results showed that either the benzopyranic-4-one plane or coplanar catecholic portion could be localized in the double helix of DNA, which depends upon the existence of foreign molecules or not.

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Received August 7, 2003; revised May 28, 2004; accepted July 1, 2004.
Project supported by the National Natural Science Foundation of China (Nos. 90206037, 20205007, 20075010).

Experimental

Chemical reagents

Double-stranded calf thymus DNA (dsDNA) was purchased from Sino-American Biotechnical Company. The purity of DNA was determined from optical measurement ($A_{260}/A_{280}>1.8$, where A represents absorbance). dsDNA was dissolved in 0.025 mol/L phosphate buffer solution (pH=7.0) prior to use. The concentration of DNA (2.8×10^{-3} mol/L in nucleotide phosphate, NP) was determined using the molar extinction coefficient of 6600 L/(mol•cm) at 260 nm. β-Cyclodextrin (β-CD, 95%, Shuzhou Gourment Factory, China) was purified by twice recrystallization in doubly distilled water. The stock solution of β-CD (1.0×10^{-2} mol/L) was prepared with doubly distilled water. Rutin was obtained from Aldrich (Milwaukee, WI, USA). Rutin (1.0×10^{-3} mol/L) was prepared with alcohol. All solutions were stored at 4 ℃.

Apparatus

Cyclic voltammetric measurements were performed with CHI660A Electrochemical Workstation (CHI Instruments Inc., USA). A glassy carbon electrode with a diameter of 4 mm was used as a working electrode, the reference electrode was a saturated calomel electrode (SCE) and the counter one was a platinum wire electrode. Absorption measurements were performed on a UV-2501PC UV-vis Recording Spectrophotometer (Shimadzu Corporation, Japan) with slit width of 1 nm. Fluorescence emission spectral measurements were performed on an RF-5301 Spectrofluorophotometer (Shimadzu Corporation, Japan). The spectra were recorded in the wavelength range of 480—600 nm by an exciting wave at 447 nm using a slit width of 10 nm. Experiments were conducted at (25.0±0.5) ℃. All reported potential values were against the SCE.

Preparation of dsDNA modified electrode

The surface of glassy carbon electrode (GCE) was polished by 0.15 μm aluminum suspension to a mirror finish. The electrode was then washed with 1∶1 HNO_3, acetone and doubly distilled water, respectively, with ultrasonic bath. The freshly-pretreated GCE was modified by transferring 2 μL of dsDNA onto its surface by air-drying. Thus dsDNA-coated GCE was obtained, denoted as dsDNA/GCE.

Results and discussion

Interactions of rutin and rutin-β-CD with DNA by cyclic voltammetry

Electrochemical method is widely used to study the interaction between DNA and small molecules. Based on the shift of formal potentials in the cyclic voltammograms, the interaction mode of foreign molecules with DNA can be inferred. When foreign molecules bind into dsDNA by imtercalation, the formal potential shifts towards more positive direction; while the electrostatic interaction, in which the foreign molecules attract electrostatically to the negative charged deoxyribose-phosphate backbone of DNA, makes the potential shift to a more negative direction.

Figure 1 showed the cyclic voltammograms of rutin at bare GCE (curve A) and dsDNA/GCE (curve B). It can be seen that, at the bare glassy carbon electrode, a couple of redox peaks for rutin appeared with a formal potential $E_{sol}^{\ominus'}$ of 0.211 V, and the difference between cathodic and anodic peak potential (ΔE_p) is 49 mV. The peak current is proportional to the square root of scan rates, as expected for a process controlled by diffusion and rate-determining. At the dsDNA/GCE, the formal potential $E_{surf}^{\ominus'}$ is 0.218 V and the ΔE_p is 50 mV. The peak current is directly proportional to scan rates, which is characteristic of a surface process. According to the shift of formal potential in the more positive direction, it can be induced that rutin can interact intercalatively with DNA.[9,11]

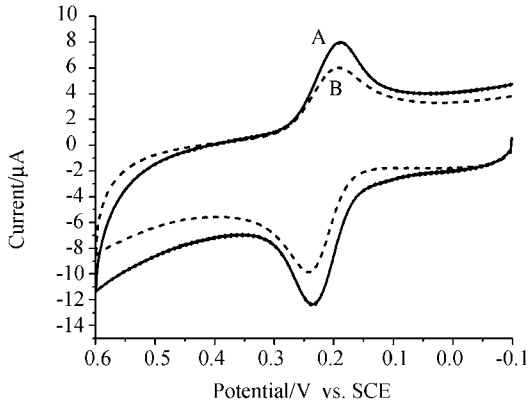


Figure 1 Cyclic voltammograms of 1.0×10^{-4} mol/L rutin in 0.025 mol/L phosphate buffer solution (pH=7.0). (A) Bare GCE; (B) dsDNA/GCE. Scan rate: 100 mV/s.

The shift of the formal potential can be also used to estimate the ratio of equilibrium constants for the binding of the reduced and oxidized forms to DNA. The general process can be described by following scheme[6,11]

$$\begin{array}{ccc} \mathrm{Rutin}_{ox,\,sol} + 2e & \overset{E_{sol}^{\ominus'}}{\rightleftharpoons} & \mathrm{Rutin}_{red,\,sol}\ \text{(rutin in solution)} \\ K_{ox}\ \Big\| & & \Big\|\ K_{red} \\ \mathrm{Rutin}_{ox,surf} + 2e & \overset{E_{surf}^{\ominus'}}{\rightleftharpoons} & \mathrm{Rutin}_{red,\,surf}\ \text{(rutin bound to electrode surface)} \end{array}$$

In such a case, one can obtain following formula[6,11]

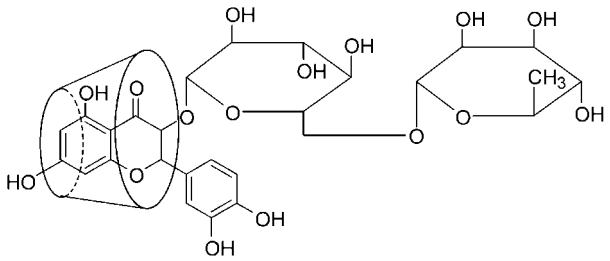
$$\Delta E^{0\,\prime}=E_{surf}^{\ominus'}-E_{sol}^{\ominus'}=-\frac{RT}{2F}\ln\frac{K_{ox}}{K_{red}} \quad (1)$$

where $E_{sol}^{\ominus'}$ and $E_{surf}^{\ominus'}$ represent the formal potential of redox species immobilized on the electrode surface and in solution, respectively; K_{ox} and K_{red} are the surface-binding constants for the oxidized and reduced

forms of the redox species, respectively. Experimental results showed that a ratio of K_{red}/K_{ox} was 1.7. This result indicated that the reduced form interacted with dsDNA on the dsDNA/GCE stronger than that of the oxidized form. Obviously, it is the evidence of intercalative interactions.[9]

In comparison with the currents of rutin at the bare GCE and dsDNA/GCE, the latter is smaller than the former. It maybe resulted from two reasons. Firstly, the active sites decreased due to the coverage of dsDNA on the electrode surface. Secondly, rutin might intercalate into DNA double helix, which resulted in the decrease of its electrochemical activity.

The cyclic voltammograms of rutin in the absence and presence of β-CD were shown in Figure 2 (curve A and B). At the GCE, there were a couple of redox peaks in the range of 0.60——0.10 V. We had ever investigated the interaction of methylene blue (MB) with DNA by means of β-CD using cyclic voltammetry.[17] The formal potential ($E^{\ominus'}$) of MB-β-CD shifted to more positive by 21 mV compared to that of MB, which meant that the planar tricyclic part of MB molecule entered the hydrophobic cavity of β-CD. As for electrochemical characteristics of rutin in this experiment, the oxidation of catecholic portion (C-ring) of rutin (shown in Scheme 1) occurred with two electrons and two protons.[20] The formal potential ($E^{\ominus'}$) of rutin-β-CD hardly changed in comparison with that of rutin alone. It manifested that the existence of β-CD did not affect the electrochemical behavior of rutin. It is inferred that the catecholic portion of rutin might be out of the cavity of β-CD, whereas the planar benzopyranic-4-one part might be included in the cavity of β-CD, which can be depicted as follows:



When DNA was added into the rutin-β-CD solution, its formal potential shifted to more positive direction by 6 mV (Figure 2C), and it probably occurred that the catecholic portion of rutin-β-CD complex may interact intercalatively with DNA. That is, the catecholic portion of rutin could intercalate into the double helix of DNA. In order to demonstrate the above assumption, the spectroscopic experiments were carried out as below.

Interaction between rutin or rutin-β-CD and DNA by UV-vis absorption spectroscopy and fluorescence emission spectroscopy

The UV-vis absorption spectroscopy is one of the most useful techniques for the study of DNA-binding.

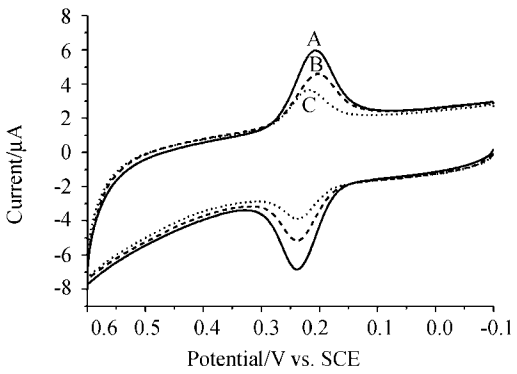
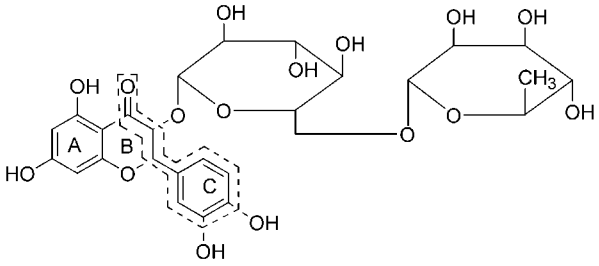


Figure 2 Cyclic voltammograms of rutin (1.0×10^{-5} mol/L) in the 0.025 mol/L phosphate buffer solution (pH=7.0). (A) Rutin only (solid line); (B) A+1.0×10^{-4} mol/L β-CD (dash line); (C) B+3.1×10^{-5} mol/L DNA (dot line).



Scheme 1 Chemical structure of rutin. Dotted frame represents the cinnamoyl part of the molecule.

As shown in figures, there were two absorption bands of rutin with the maximum wavelength of 352.6 (band I) and 252.6 nm (band II) respectively. With the increase of DNA concentration, a bathochromic shift and hypochromism of band I were obviously observed, and it is like the phenomenon yielded by the interaction between DNA and its typical intercalator.[21] Based on the variations of absorbance at the band I, the binding constant, K, of rutin-DNA complex can be obtained according to the following equation: [19]

$$\frac{A_0}{A-A_0}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K[\mathrm{DNA}]} \qquad (2)$$

where A_0 and A are the absorbance of the free guest and the apparent one, ε_G and ε_{H-G} are their absorption coefficients, respectively. The result by fitting the experimental data with Eq. (2) was shown in inset of Figure 3. It suggested that the complex of rutin with DNA (per nucleotide phosphate) is to be a kind of 1∶1 complex. From the plot of $A_0/(A-A_0)$ to 1/[DNA], the ratio of the intercept to the slope gives the binding constant, $K=2.9\times10^4$ L/mol.

In order to clarify the portion of rutin interacting with DNA, the inclusion complex of rutin-β-CD was used to further study the interaction site by spectroscopic method. The UV-vis spectra of rutin were recorded in the absence and presence of β-CD (shown in Figure 4). Comparing curve 1 to 2, the shift of band I towards longer wavelengths for 4 nm was observed, and

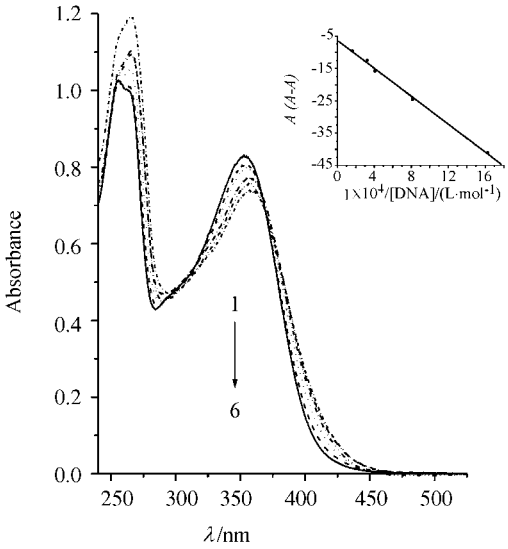


Figure 3 Effect of DNA on rutin UV-vis spectra in 0.025 mol/L phosphate buffer solution (pH=7.0). [rutin]=5.0×10^{-5} mol/L. Curves 1—6: DNA concentrations are 0.00, 6.2 , 12.4, 24.8, 31.0 and 62.0 μmol/L, respectively. Inset: The plot of $A_0/(A-A_0)$ versus 1/[DNA].

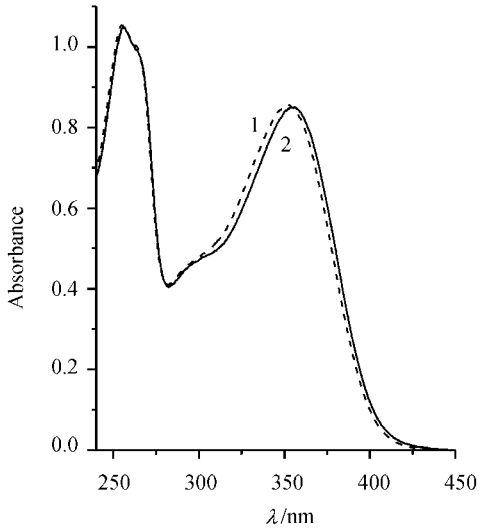


Figure 4 Absorption spectra of rutin (5.0×10^{-5} mol/L) in the absence (curve 1) and presence (curve 2) of β-CD (2.0×10^{-3} mol/L) in 0.025 mol/L phosphate buffer solution (pH=7.0).

the absorbance hardly changed. It revealed that the inclusion complex of rutin with β-CD was formed. Due to more hydrophobic with a regulation planarity of benzopyranic-4-one plane than that of the cinnamoyl system,[22] benzopyranic-4-one plane is easier to interact with β-CD, and the inclusion complex is formed. In the UV-vis spectrum of rutin, the band I was supposed to be associated with the absorption of the cinnamoyl system (shown in dotted frame of Scheme 1).[22] Because of the B-ring localizing in the cavity of β-CD, it also affected the absorption of the cinnamoyl system. So the band I shifts to longer wavelength in the presence of β-CD. Likewise, based on the shift to longer wavelength at band I in Figure 3 in the presence of DNA, the benzopyranic-4-one of rutin was apt to intercalate into the double helix of DNA.[13]

As shown at the curves a and c in Figure 5, the fluorescence intensities of rutin increase obviously with the increase of β-CD concentration (curves a and c). It resulted from that the cavity of β-CD offers a protective microenvironment shielding the excited single species from quenching and nonradiative decay process occurring in solution. This fact further manifested that the inclusion complex of rutin with β-CD was formed. At the same time, the association constant K of rutin with β-CD was determined to be 580 using steady state fluorescence method.[16] When DNA was added to rutin solution, the fluorescence intensities increased markedly (Figure 5, curve b). It resulted from the increase of fluorescence quantum yield when rutin intercalated into the hydrophobic environment of the inner DNA-bases. When DNA was in the rutin solution containing 1.0×10^{-3} mol/L β-CD (Figure 5, curve d), the fluorescence intensity still markedly increased. Comparing curve b and d, it was inferred that the inclusion complex of rutin-β-CD is not decomposed by DNA but combines with DNA. It revealed that the ternary complex was formed.

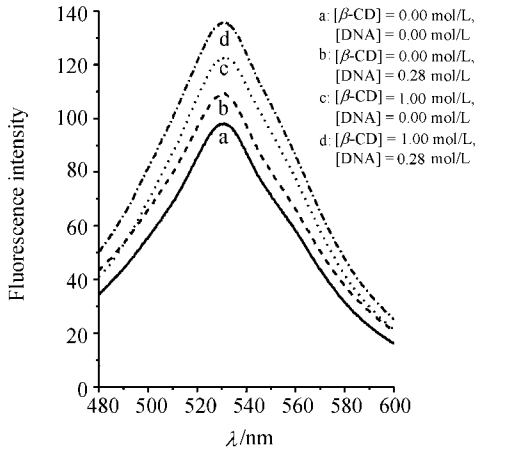


Figure 5 Fluorescence emission spectra of 1.0×10^{-4} mol/L rutin in the absence and presence of β-CD and DNA (pH=7.0).

When DNA was in the rutin and β-CD-rutin solutions respectively, an appearance of a fluorescence band in the region 480—600 nm with a maximum emission at 530 nm was observed, and the fluorescence intensity increased with the increase of DNA concentration in rutin and rutin-β-CD solutions (shown in Figure 6). It revealed that the interactive model between rutin-β-CD and DNA is the same as that between rutin and DNA. It showed that the catecholic portion of rutin-β-CD is located in the double helix of DNA. The results are in good agreement with those obtained by electrochemical method.

According to the experimental results, a conclusion is drawn that the interaction sites of small molecules with DNA can be adjusted by the formation of a β-CD host-guest compound.

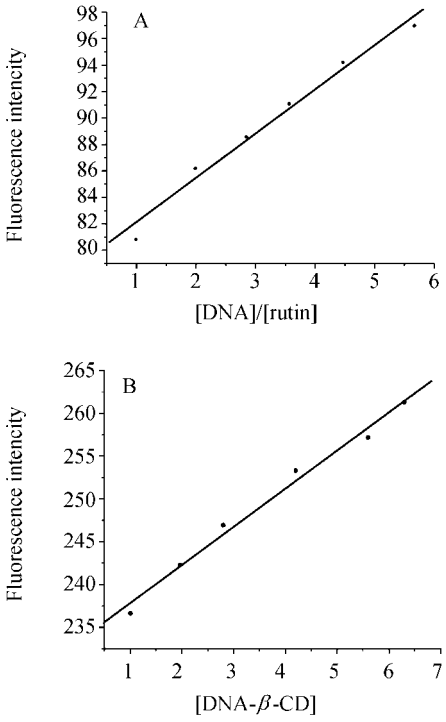


Figure 6 Plots showing fluorescence intensity at 530 nm vs. [DNA]/[rutin] (A) and [DNA]/[rutin-β-CD] (B). (A): [rutin]=1.0 $\times10^{-4}$ mol/L; (B): A+5.0$\times10^{-3}$ mol/L β-CD and DNA (pH= 7.0).

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(E0308078 LU, Y. J.)