

Study of Inclusion Complexes of Cyclodextrins with Orange II

PEIQING ZHENG, ZHEN LI, LINHUI TONG and RUNHUA LU*

State Key Laboratory for Oxo Synthesis and Selective Oxidation, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, P.R. China

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Abstract

Inclusion reactions of α - and β -CD with Orange II were measured by means of a stopped-flow method and UV-spectra. The forward rate constants (k_+), backward rate constants (k_-) and the equilibrium constants (K) for the inclusion reactions were determined. The inclusion processes of CD with Orange II follow a one-step reaction mechanism. The value of the forward rate constant at neutral and alkaline conditions for Orange II binding to α -CD cavity was large. This suggests that the naphthalene side is partially included in the final complex form. In the case of β -CD, only marginal difference is shown. It is probable that inclusion covers the whole naphthalene group and part of the azo group as well, resulting in a small effect from the pH variation. The results show that the equilibrium constant at pH 7 for the binding of Orange II to α -CD is the largest because the included part of Orange II matches the cavity of α -CD well.

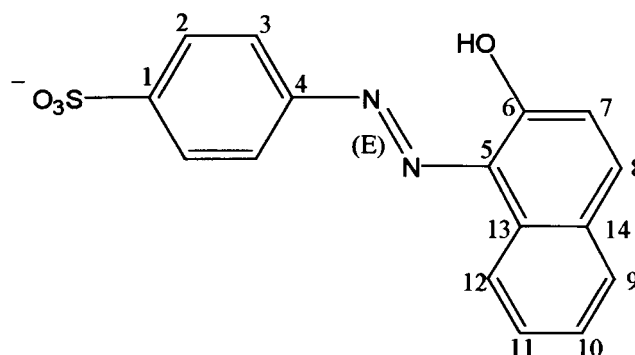
Introduction

Native cyclodextrins (CDs) are cyclic oligosaccharides composed of six (α -), seven (β -), or eight (γ -) α -1,4-linked D-glucopyranose residues. They form inclusion complexes with a lot of azo dyes. Among those compounds Orange II (Scheme 1) is the first selected for studying because of its two-set substrate. Over the last decade, UV, circular dichroism and ^1H , ^{13}C NMR spectra have been used to study CD-Orange II complexes and valuable information such as the stability constant, inclusion direction and position within cyclodextrin has been obtained [1–7]. Up to now a few papers about kinetics have been reported. Nevertheless, the study of dynamic features is essential to clarify the driving force and the reaction mechanism for the molecular recognition by cyclodextrins [9]. In the present paper, both UV and stopped-flow techniques were used to estimate the equilibrium and kinetic rate constants at different pH. The results obtained in this experiment have been compared with previous works and the direction as well as the inclusion state of Orange II within CD cavities have been discussed.

Experiment

Materials

α -CD was a gift from professor Linhui-Tong. β -CD (95%, Suzhou Weijing Plant, China). It was recrystallized three times in distilled water and subsequently dried in vacuum at 100 °C for 24 hours. Orange II and other materials used



Scheme 1.

were analytical reagent grade. The pH of the buffer solution was adjusted manually using sodium hydroxide and sodium carbonate as appropriate.

UV-vis spectra

Stability constants of the complexation of α - and β -CD with Orange II in alkaline (pH 9.4, Na_2CO_3 — NaHCO_3), and neutral (pH 7.0, KH_2PO_4 — NaOH) conditions were determined at ambient temperature from spectrophotometric titrations with a Shimadzu UV-240 spectrophotometer equipped with OPI-4 program. Aqueous solutions of Orange II (1×10^{-5} M) was then titrated with α -CD or β -CD ($1 \times 10^{-4} \sim 1.8 \times 10^{-3}$ M) with a large excess of the dye.

Kinetic measurements

Kinetic measurements were carried out on a biosequential stopped-flow ASVD spectrofluorimeter DX-17MV equipped with optical detection and a data processor. The

* Author for correspondence.

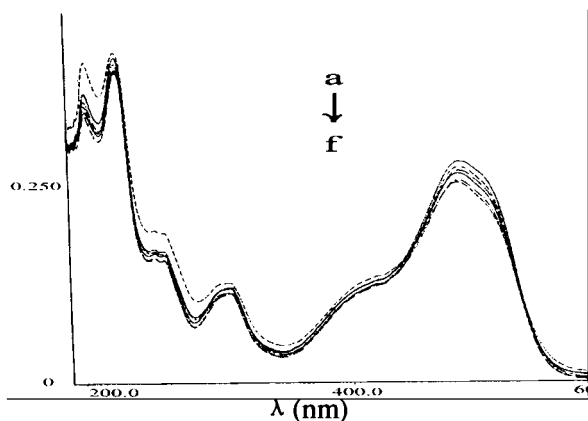


Figure 1. Absorption spectra for complexes of Orange II and α -cyclodextrin at pH 9.4. a. $[G] = 1 \times 10^{-5}$ M, b. $[\alpha\text{-CD}]_0:[G]$ 10:1, c. 20:1, d. 60:1, e. 80:1, f. 180 = 1.

data collected gave absorbance-time ($A-t$) and absorbance-wavelength ($A-\lambda$) curves after autoanalysis and a single exponential curve was obtained simultaneously. The concentration of Orange II was fixed at 2×10^{-5} M, while CD was varied at 4×10^{-4} – 5×10^{-3} M. In the experiment, the solution of CD was rapidly mixed at room temperature with an equal volume of Orange II solution and a fitting method was used to calculate the observed rate constant [9].

Results and discussion

UV-spectra

The absorbance changes which occur when Orange II binds to α -cyclodextrin at pH 9.4 are shown in Figure 1. The same behavior is found for all other complexes. The maximum absorption at 482 nm is attributed to the long wavelength $\pi-\pi^*$ transition of Orange II. The isobestic point indicates the formation of precisely 1:1 complexes. The equilibrium constants (K_{spec}) were determined from the spectra using a modified Benesi-Hildebrand equation [8]

$$1/\Delta A = 1/K\alpha[CD]_0 + 1/\alpha, \quad (1)$$

where ΔA is the difference in absorbance between the presence and absence of CD and α is a constant related to the experimental conditions. A typical plot of $1/\Delta A$ versus $1/[CD]_0$ is shown in Figure 2. Other experimental results for α - and β -CD at pH 7.0 or pH 9.4 obtained are all of a linear variation ($1/\Delta A = f(1/[CD]_0)$). The values of K_{spec} obtained are collected in Table 2.

The value of the K_{spec} of the α -CD-Orange II complex in neutral solution is higher than in an alkaline solution, but the case of β -CD-Orange II complex is contrary. It illustrates that the inclusion state for α -CD is different from β -CD.

Kinetic studies

The kinetics of the inclusion reactions were investigated by a stopped-flow apparatus which is equipped in the absorption mode for detection. The experimental results indicate that a

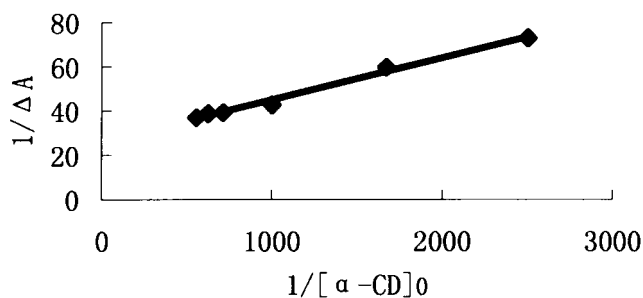


Figure 2. Plots of $1/\Delta A$ versus $1/[\alpha\text{-CD}]_0$. For a mixture of Orange II and α -CD at pH 9.

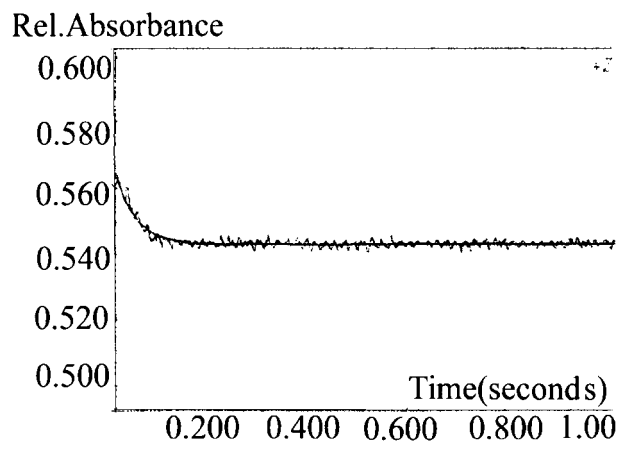
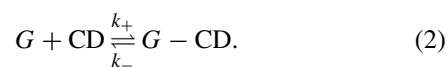


Figure 3. Stopped-flow signal at 482 nm for the formation of Orange II/ α -CD inclusion complex at pH = 7.0.

detectable spectral change in the UV-vis region between the free and the complexed guest molecule were obtained. Typical stopped-flow signal observed at 482 nm for the inclusion reaction of the acid form (pH = 7.0) of Orange II with α -CD is illustrated in Figure 3. The decay curve in absorbance is exponential in the process and a resolvable absorption change is clearly observed. The experiments were performed under pseudo-first order conditions in the presence of excess of α - and β -CD. The rate data are found to obey the following one-step mechanism (where $K_{\text{kin}} = k_+/k_-$) [9]:



Under the conditions of the experiment, values of k_+ and k_- are then obtained from the observed relaxation time τ ($k_{\text{obs}} = \tau^{-1}$):

$$\tau^{-1} = k_+[CD]_0 + k_- [9].$$

The plot of k_{obs} versus the total concentration of the host ($[CD]_0$) is shown in Figure 4.

The plot gives a straight line which indicates that the molar ratio of the host/guest system is 1:1. From the slope (k_+) and the intercept (k_-), the equilibrium constant (K_{kin}) is calculated (Table 1).

The rate constant of the inclusion process of α -CD with Orange II decreases with the increase of pH while that of β -CD with Orange II shows a small increase. The results indicate that the equilibrium and rate constants of Orange

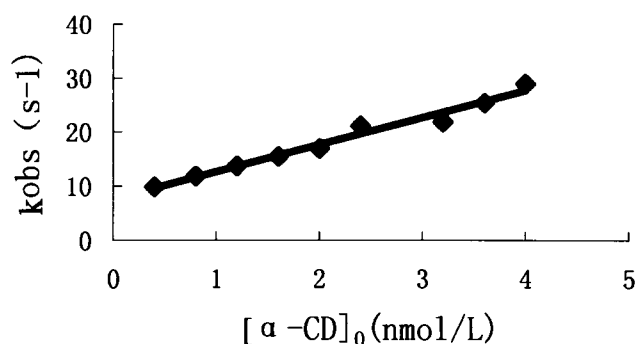


Figure 4. Plot of the observed kinetic rate versus initial concentration of α -CD at pH 7.0 for complexes of Orange II/ α -CD.

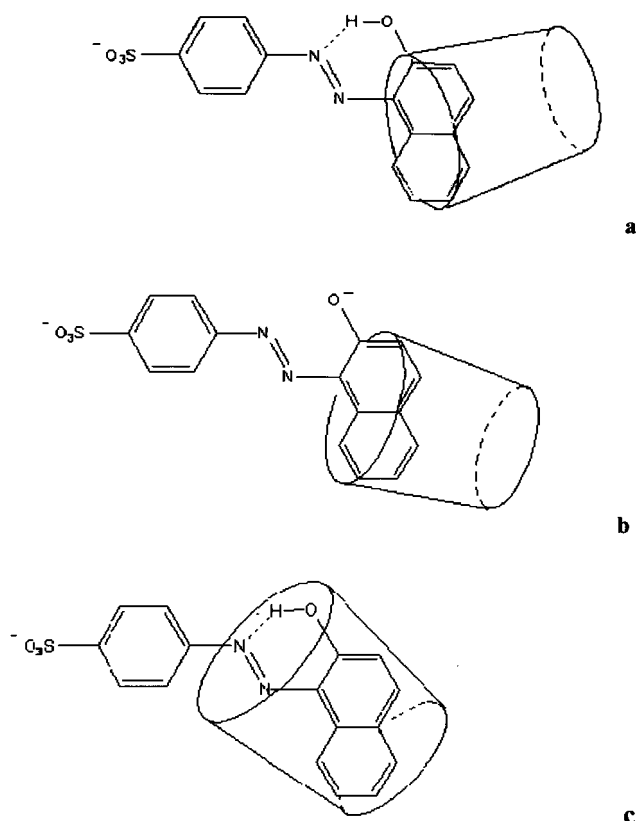
Table 1. Equilibrium and rate data for the Orange II binding to CD determined kinetically

pH	Host	K_{+1} (mol ⁻¹ dm ³ s ⁻¹)	k_{-1} (s ⁻¹)	K_{kin} (mol ⁻¹ dm ³)
7.0	α -CD	5004	7.7	652
7.0	β -CD	1319	9.7	136
9.4	α -CD	1398	12.4	113
9.4	β -CD	2144	11.0	195

II/ α -CD are the largest when pH is 7.0. For the direction of recognition, it seems that only the benzenesulphonate group of the guest molecule can be included into the α -CD cavity since the dimension of the naphthalene nuclei is too large to be fit for the cavity of the α -CD molecule. However, the ratio values of k_+ and k_- are relatively large at neutral solution and only one-step inclusion can be detected. This fact suggests that Orange II can be readily embedded in the α -CD cavity with a small blocking. In addition, a relatively significant decrease in the association rate constant with the increase of pH expresses that the inclusion takes place from the naphthalene side. Corey–Pauling–Koltum (CPK) models show that the second side of the α -CD cavity can partially include the naphthalene nucleus side of Orange II (Scheme 2a,b). On the other hand, the ring protons (H-9, H-10) of Orange II show selective low field shifts but the H-3 proton of α -CD shows high field shifts due to the ring current effect. The C-1 and C-4 positions of α -CD show low field shifts which may be due to changes of the bond angle of

Table 2. Comparison of equilibrium constants obtained by spectrophotometry (K_{spec}) and stopped-flow kinetics (K_{kin})

pH	Host	K_{kin} (mol ⁻¹ dm ³)	K_{spec} (mol ⁻¹ dm ³)
7.0	α -CD	652	4006
7.0	β -CD	136	278
9.4	α -CD	113	1389
9.4	β -CD	195	2558



Scheme 2.

the glycosidic linkage loop arrangement. In marked contrast to α -CD is the fact that the Orange II- β -CD complex shows that the stability and rate constants are larger in an alkaline solution than in a neutral condition. CPK models indicate that β -CD can include almost the whole naphthalene nucleus side or the benzenesulphonate side of Orange II. ¹H, ¹³C NMR spectra both indicate that Orange II is affected by the hydrophobic cavity of the β -CD [2, 3]. The molecular motions of the β -CD complex with Orange II have been studied on the basis of ¹³C-T1 measurements [10]. This expresses that the complex still permits the Orange II molecule to rotate in the cavity. The relatively loose binding results in a lower stability constant and the electrostatic interaction between O⁻ of naphthalene group and CD may be minimal. CD spectra should add further information to aid in determining from which direction inclusion occurs. In the β -CD complex with Orange II the π – π^* band showed positive splitting [2, 5]. On the other hand, reversed signals of the CD spectrum in the β -CD complex indicate that N=N is included from a different direction than others (Scheme 2c). In our opinion, it is the naphthalene nucleus side that may insert into the cavity from the second side of α -CD. The included part of Orange II matches the cavity of α -CD well. Furthermore, when pH is 7.0, the H atom of hydroxy on Orange II did not dissociate and N=N was not protonated, so the complexation process of guest binding to host is the fastest when the host is α -CD and when pH is 7.0.

Conclusion

Inclusion reactions of α - and β -CD with Orange II were measured by means of a stopped-flow method and UV-technique. The forward rate constants (k_+), backward rate constants (k) and the equilibrium constants (K) of the host with Orange II were determined. The linear relationship between the concentration of host and the observed rate data (k_{obs}) indicates that the molar ratio of the host/guest system is 1:1. The inclusion process of CD with Orange II follows a one-step reaction mechanism. Data obtained from the stopped-flow method and UV as well as NMR are rationalized in terms of inclusion direction of the naphthalene side of the guest azo molecule along the short axis into the cavity of both α - and β -CD.

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