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An equilibrium study of *p*-Methyl Red inclusion complexes with α- and β-cyclodextrins

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ABSTRACT

The effects of cyclomaltohexaose (α-cyclodextrin, αCD) and cyclomaltoheptaose (β-cyclodextrin, βCD) on the UV–visible spectra of the anion and cation of *p*-Methyl Red {4-[4-(dimethylamino)phenylazo]benzoic acid} in aqueous solution were analyzed according to 1:1 inclusion processes. The values of the thermodynamic parameters ΔH^0, ΔS^0, and ΔG^0 were calculated from the effect of temperature on the thermodynamic stability constants of the inclusion complexes of the anion and cation with both cyclodextrins. The stability constants of the inclusion complexes of the tautomers of *p*-Methyl Red were evaluated at 25°C. The addition of a cyclodextrin shifts the position of the tautomeric equilibrium towards the side of the ammonium tautomer. Both α- and β-cyclodextrin bind the ammonium tautomer more strongly than the azonium tautomer. The most stable inclusion complex is that of the anion with αCD; the other complexes are of comparable stability.

INTRODUCTION

The inclusion phenomenon exhibited by cyclomalto-oligosaccharides (cyclodextrins) has been much investigated because of its relevance to enzyme–substrate and drug–receptor interactions[1,2]. The binding of a molecule or ion to the cavity of a cyclodextrin results in the formation of an inclusion complex that involves relatively weak nonspecific interactions[3]. The most common cyclodextrins are cyclomaltohexaose (α-cyclodextrin, αCD), cyclomaltoheptaose (β-cyclodextrin, βCD), and cyclomaltooctaose (γ-cyclodextrin, γCD), which are cyclic oligomers of D-glucose containing six (αCD), seven (βCD), and eight (γCD) D-glucosyl residues. The size of a cyclodextrin cavity is governed by the number of D-glucosyl residues. The interior of the cavity provides a hydrophobic microenvironment while its exterior side exposes a hydrophilic surface with the secondary hydroxyl groups forming the wider rim and the primary hydroxyl groups forming the narrower rim of the cavity. Several kinetic and equilibrium studies have been reported on the

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inclusion complexes of cyclodextrins with azo dyes of variable structural complexities[3–5]. Among the relatively simple azobenzene dyes, the inclusion complexes of Methyl Orange with cyclodextrins have been much investigated[4–10]. To the best of our knowledge, only one equilibrium study has been reported on the binding of *o*-Methyl Red {2-[4-(dimethylamino)phenylazo]benzoic acid} to cyclodextrins[9]. However, *p*-Methyl Red has been investigated in connection with its *cis–trans* photoisomerization in the host–guest Langmuir–Blodgett films prepared with amphiphilic β-cyclodextrin derivatives[11,12].

The purpose of the present study was to investigate the binding of *p*-Methyl Red anion and cation to α- and β-cyclodextrins in aqueous solutions, and to report the thermodynamic parameters of the inclusion processes of *p*-Methyl Red. The absence of such information in the literature motivated us to carry out this study.

EXPERIMENTAL

The acid form of *p*-Methyl Red ($C_{15}H_{15}N_3O_2$) was prepared according to a procedure similar to that suggested for the synthesis of *o*-Methyl Red[13,14]. The details of the preparation are given elsewhere[15,16]. The purified sample of *p*-Methyl Red had a melting point of 266°C. Anal. calcd for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60. Found: C, 67.03; H, 5.54; N, 15.75. The ^1H NMR spectrum of the *p*-Methyl Red sample was consistent with the above formula[16]. The test sample was dried at 130°C for 10 h before use. The samples of αCD and βCD were purchased from Sigma (St. Louis, MO, USA) and were used without further purification. The required amount of a cyclodextrin was weighed in the hydrated form and the concentration was calculated on a dry basis[10]. Other chemicals used in this study were reagent grade. Carbonate-free NaOH solutions were prepared as suggested by Vogel[17].

A typical stock solution of *p*-Methyl Red had a concentration of 3.97×10^{-4} mol dm^{-3}, an ionic strength of 7.10×10^{-4} mol dm^{-3}, and a pH value of 10.5. Under these conditions, *p*-Methyl Red is present as the sodium salt. Stock solutions of ca. 6.0×10^{-3} mol dm^{-3} of αCD and βCD were used. Solutions needed for studying the inclusion complexes of the anion of *p*-Methyl Red (structure A, Fig. 1) were prepared by using a fixed amount of *p*-Methyl Red stock solution and varying the amounts of αCD or βCD stock solution. In a typical experiment, 1.00 mL of the *p*-Methyl Red stock solution was transferred to a 25-mL volumetric flask, followed by adding the required amount of the cyclodextrin stock solution and 0.40 mL of 8×10^{-3} mol dm^{-3} NaOH solution to attain a pH value of 9.5 and an ionic strength of 1.50×10^{-4} mol dm^{-3}. The same procedure was followed for studying the inclusion complexes of the *p*-Methyl Red cation at pH 1.0, with the exception that HCl and NaCl solutions were used. NaCl was used to vary the ionic strength in the range 0.10–1.0 mol dm^{-3}. The final concentration of *p*-Methyl Red (at pH 9.5 or 1.0) was fixed at 1.586×10^{-5} mol

dm^{-3} while the concentration of either αCD or βCD was varied in the range 1.0×10^{-4}–4.0×10^{-3} mol dm^{-3}. The UV–visible spectra of the test solutions were recorded at 16.0, 25.0, 33.0, and 40.5°C, using a double-beam spectrophotometer (DMS 100, Varian) and a quartz cell with optical path length of 1.00 cm. Other experimental details are the same as those given previously[10].

RESULTS AND DISCUSSION

The UV–visible spectrum of the *p*-Methyl Red anion shows two absorption maxima at 464 and 273 nm, which were assigned as π–π^* and n–π^* transitions of the azo linkage and the carboxyl group, respectively[18]. The addition of αCD to the aqueous solution of the *p*-Methyl Red anion resulted in a decrease in the intensity of the absorption at 464 nm and a blue shift of ca. 20 nm in the absorption maximum accompanied by the formation of an isosbestic point at 450 nm. The absorptivity at 273 nm also decreased, due to the addition of αCD but the absorption maximum remained unchanged. The effect of adding βCD on the absorption in the visible region was similar to that of αCD except that the blue shift in the absorption maximum was ca. 9 nm. However, βCD had no measurable effect on the absorptivity at 273 nm.

In 0.10 mol dm^{-3} HCl, *p*-Methyl Red is considered to be in the cationic diprotonated form[18], which is composed of the azonium and ammonium tautomers as shown in Fig. 1. The UV–visible spectrum of the *p*-Methyl Red cation shows two absorption maxima at 510 and 320 nm. The absorption at 510 was attributed to the azonium tautomer (the resonance hybrid of structures C and D, Fig. 1) while that at 320 nm was attributed to the ammonium tautomer (structure B, Fig. 1). This assignment was based on literature information concerning the spectra of azobenzene dyes in acidic solutions[19]. The addition of αCD to the aqueous

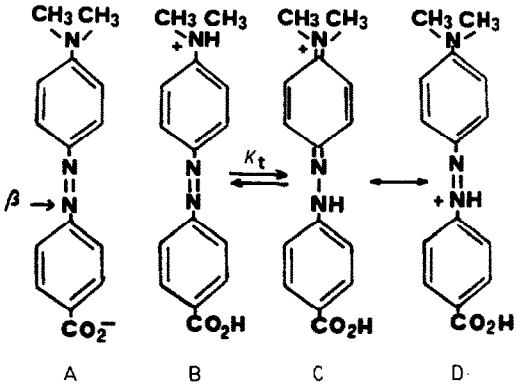

Fig. 1. Structural formulas of the anion (A) and the cation of *p*-Methyl Red. The symbol B stands for the ammonium tautomer (am), while the symbols C and D stand for the resonance structures of the azonium tautomer (az).

solution of the *p*-Methyl Red cation resulted in a blue shift of ca. 3 nm in the absorption maximum of the azonium tautomer and a red shift of ca. 10 nm in the absorption maximum of the ammonium tautomer. However, the intensity of the absorption of the azonium tautomer decreased and that of the ammonium tautomer increased with increasing concentration of αCD. βCD had the same effect as αCD except that the position of the absorption maximum for both tautomers did not change with increasing concentration of βCD. These spectral observations were interpreted to be a result of the occurrence of an inclusion process where the anion or the cation of *p*-Methyl Red binds to a cyclodextrin cavity. In principle, more than one stoichiometry can be proposed for the binding of *p*-Methyl Red to a cyclodextrin cavity. In addition, the *cis–trans* isomerism of the azo linkage and the tautomerism of the cation require the consideration of several inclusion complexes for a given stoichiometry. The investigation of all these possibilities was not feasible in the present study.

In relatively dilute aqueous solutions of azo dyes and cyclodextrins, the common assumption is to propose a 1:1 inclusion process which can be represented by eq *1*,

$$D + CD \overset{K_f}{\rightleftharpoons} D \cdot CD \qquad (1)$$

where the symbols D, CD, D · CD, and K_f stand for the anion or cation of *p*-Methyl Red, αCD or βCD, the inclusion complex, and the stability constant, respectively. The value of the stability constant can be obtained from the spectrophotometric data by considering the Benesi–Hildebrand equation in the following form[10]

$$l \cdot C_o \cdot S_o/\Delta A = (1/K_f \cdot \Delta\epsilon) + C_o/\Delta\epsilon \qquad (2)$$

where l is the optical path length of the cell used, C_o and S_o represent the initial molar concentrations of a cyclodextrin and *p*-Methyl Red, ΔA is the change in the absorbance of *p*-Methyl Red due to the addition of a cyclodextrin, and $\Delta\epsilon$ is the difference in the molar absorptivities between free and complexed *p*-Methyl Red. Equation *2* describes a 1:1 stoichiometry and is a straight-line equation with slope equal to $1/\Delta\epsilon$ and intercept equal to $1/K_f \cdot \Delta\epsilon$. The ratio slope/intercept provides a value for the stability constant at a given wavelength either graphically or by using a linear least-squares analysis of the absorbance data.

The spectrophotometric data obtained from the effect of αCD and βCD on the visible spectrum of the anion of *p*-Methyl Red at pH 9.5 were found to be consistent with eq *2*. The stability constant was found to be wavelength-independent, since the deviations from the mean of a set were less than $\pm 3\%$. The same arguments are applicable for the inclusion process of the *p*-Methyl Red cation. However, the stability constants of the cation complexes were found to be dependent on the ionic strength of the solution. This dependence will be discussed later.

For both the anion and the cation complexes, the value of the ratio slope/intercept of eq *2* was found to be independent of the initial concentration of

p-Methyl Red. This evidence supports our assumption of the 1:1 stoichiometry as given by eq *1*. It should be pointed out that a modification of eq *2* has been suggested for evaluating the stability constant of a 2:1 (dye–cyclodextrin) inclusion complex[20] and the inspection of such an equation reveals that the value of the ratio slope/intercept depends on the initial concentration of the azo dye. In fact, our work on the binding of *p*-Methyl Red to γ-cyclodextrin (to be published separately) indicates that the stoichiometry of the inclusion process is of the 2:1 type. It seems that the cavity of either αCD or βCD cannot accommodate a dimer of *p*-Methyl Red anion or cation as does the larger cavity of γCD. The anion of Methyl Orange was reported to bind to γCD according to a 2:1 stoichiometry[4]. The binding of the first acid conjugate of Methyl Yellow and Methyl Orange to αCD and βCD was reported to be according to a 1:1 inclusion process[10].

Based on previous arguments[10], the stability constants of the inclusion complexes of the individual tautomers of the *p*-Methyl Red cation with αCD and βCD can be evaluated at 25°C by considering the following equilibria:

$$\begin{array}{ccc} \mathrm{az} + \mathrm{CD} & \overset{K_{az}}{\rightleftharpoons} & \mathrm{az}\cdot\mathrm{CD} \\ \Big\updownarrow K_t & & K^* \Big\updownarrow \\ \mathrm{am} + \mathrm{CD} & \underset{K_{am}}{\rightleftharpoons} & \mathrm{am}\cdot\mathrm{CD} \end{array} \qquad (3)$$

where K_{am} and K_{az} are the stability constants of the ammonium complex and the azonium complex, respectively, K^* is the tautomeric equilibrium constant of the tautomerism of the az · CD and am · CD complexes while K_t is the constant that describes the tautomerism in the absence of a cyclodextrin. It can be shown that

$$K_{az}/K_{am} = K^*/K_t \qquad (4)$$

The values of K_{am} and K_{az} can be obtained by solving the following two equations[10].

$$K_{am} = K_f(1 + K_t)/(1 + K^*) \qquad (5)$$

$$K_{az} = K_f(1 + K_t)(K^*/K_t)/(1 + K^*) \qquad (6)$$

According to the procedure suggested for calculating K^* from the absorbance data in the UV region[10] (near $\lambda = 320$ nm), the values of K^* at 25°C are 1.1 and 1.7 for the binding of the *p*-Methyl Red cation with αCD and βCD, respectively. The value of K_t is 4.0 at 25°C (ref 18). The values of K_f at 25°C and ionic strength of 0.10 mol dm^{-3} are 1.55×10^3 and 1.12×10^3 for the inclusion complexes of the cation with αCD and βCD, respectively. Substituting these values into eqs *5* and *6* yields the following values of K_{am} and K_{az} at 25°C. For the binding of the ammonium and azonium tautomers to αCD, K_{am} is 3.7×10^3 mol^{-1} dm^3 and K_{az} is 1.0×10^3 mol^{-1} dm^3. The corresponding values for binding to βCD are 2.1×10^3 mol^{-1} dm^3 and 8.8×10^2 mol^{-1} dm^3 for K_{am} and K_{az}, respectively. The

evaluation of K_{am} and K_{az} at temperatures other than 25°C is not feasible because the molar absorptivities of the model compounds for the two tautomers are not available[10].

The values of K_{am} and K_{az} indicate that αCD binds the tautomers of *p*-Methyl Red stronger than does βCD. This finding is in accord with the values of K_f which describes an overall inclusion process with both cyclodextrins. It seems that the relatively smaller cavity of αCD provides a better fit for the inclusion of the cation than does the relatively larger βCD cavity. The fact that $K_{am} > K_{az}$ for both αCD and βCD complexes can be explained according to an argument given previously for the binding of the tautomers of Methyl Orange and Methyl Yellow with αCD and βCD[21]. The localization of H^+ on the azo linkage (most likely on the β-nitrogen) in the azonium tautomer makes the penetration of the azonium tautomer less than that of the ammonium tautomer because of the association of water molecules around the azo linkage. Such association is not present in the case of the ammonium tautomer, because H^+ is attached to the nitrogen of the dimethylaniline group which is assumed not to be the penetrating side of the cation[21]. The values of K^* are less than that of K_t, indicating that the addition of either αCD or βCD shifts the tautomeric equilibrium of *p*-Methyl Red in favour

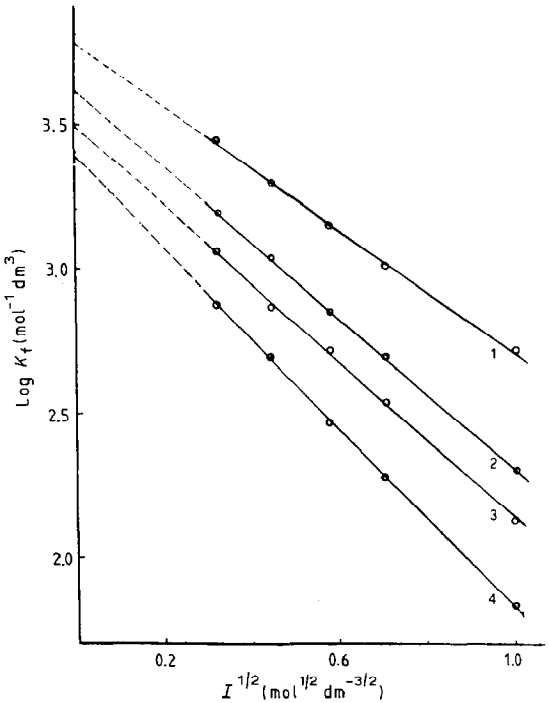

Fig. 2. The effect of ionic strength on the values of K_f for the inclusion of the *p*-Methyl Red cation by αCD. The sequence 1–4 represents data obtained at 16.0, 25.0, 33.0, and 40.5°C, respectively.

of the ammonium tautomer, i.e., stronger complexation is achieved by the ammonium tautomer. A similar finding was reported for the binding of Methyl Orange and Methyl Yellow with both αCD and βCD[10].

In order to evaluate the thermodynamic parameters of the inclusion processes of *p*-Methyl Red with αCD and βCD, the effect of ionic strength on K_f was considered, according to the following equation:

$$K = K_f \cdot K_\gamma \tag{7}$$

where K is a thermodynamic stability constant and K_γ is the ratio of the activity coefficients of the species participating in the reaction given by eq *1*. K_γ is affected by temperature and ionic strength. For the inclusion complexes of the anion of *p*-Methyl Red, K_γ is assumed to be unity since the ionic strength was ca. 1.5×10^{-4} mol dm^{-3}. However, for the inclusion complexes of the cation, the lowest value of the ionic strength was 0.10 mol dm^{-3} because of the amount of HCl required to have *p*-Methyl Red as a cation in solution. Figs. 2 and 3 show the effect of ionic strength, I, on K_f at four temperatures where I is in the range $0.10 \leq I \leq 1.0$ mol dm^{-3}. At each temperature, the value of K for a given system can be obtained by extrapolation to zero ionic strength (actual values were

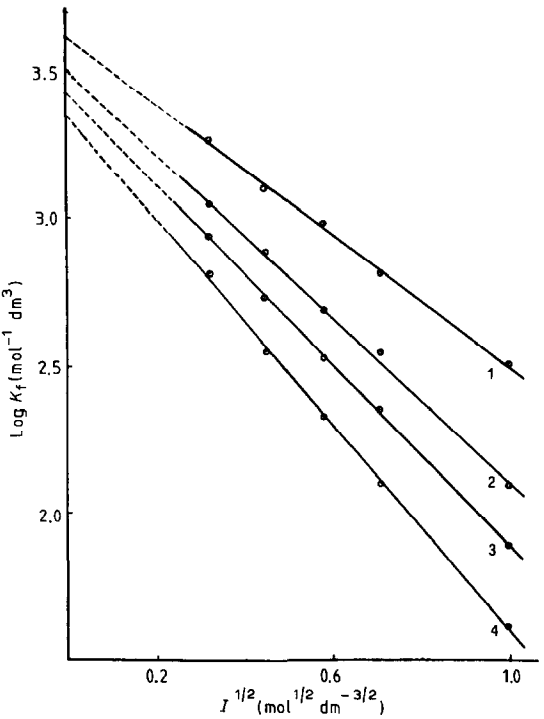

Fig. 3. The effect of ionic strength on the values of K_f for the inclusion of the *p*-Methyl Red cation by βCD. The sequence 1–4 represents data obtained at 16.0, 25.0, 33.0, and 40.5°C, respectively.

TABLE I

Values of the thermodynamic stability constants of the inclusion complexes of the anion and cation of *p*-Methyl Red with αCD and βCD at different temperatures

Temperature (°C)	K (10^3 mol^{-1} dm^3)			
	Anion complexes [a]		Cation complexes [b]	
	αCD	βCD	αCD	βCD
16.0	12.4 ±0.30	5.10±0.10	5.81±0.15	4.09±0.25
25.0	9.06±0.22	3.81±0.11	4.06±0.11	3.19±0.21
33.0	7.63±0.03	3.23±0.06	3.08±0.14	2.63±0.05
40.5	6.22±0.10	2.67±0.03	2.38±0.07	2.21±0.10

[a] The uncertainties are standard deviations. [b] The uncertainties were obtained from the error estimates of the intercepts of Figs. 2 and 3.

obtained from the intercept of the linear least-squares analysis of the data of log K_f vs. $I^{1/2}$). The values of K at four temperatures are given in Table I for the inclusion complexes of αCD and βCD with the anion and cation of *p*-Methyl Red. Figs. 2 and 3 indicate that K_f decreases as I is increased. However, Matsui and Mochida[8] reported the opposite for the binding of the first conjugate acid and the anion of Methyl Orange to αCD, and concluded that the hydrophobic rather than the electrostatic interactions play a dominant role in the association of cyclodextrins with dyes. On the other hand, Smetana and Popov[22] examined the influence of ionic strength on the equilibrium constant (defined in terms of concentrations) of an ion-molecule reaction (of the type given in eq *1*) and concluded that the equilibrium constant remained reasonably constant for $I \leq 0.05$ mol dm^{-3} and begins to decrease at higher ionic strengths. This finding is in accord with our data as given in Figs. *2* and *3*.

The values of K given in Table I were subjected to a linear least-squares analysis according to the following thermodynamic equation:

$$(2.303)R \log K = -\Delta H^0/T + \Delta S^0 \qquad (8)$$

Table II shows our results for the thermodynamic parameters ΔH^0, ΔS^0, and ΔG^0 for the inclusion complexes of αCD and βCD with the anion (MR^-/αCD and

TABLE II

Values of ΔH^0, ΔS^0, and ΔG^0 for the inclusion complexes of the anion (MR^-) and the cation (H_2MR^+) of *p*-Methyl Red with αCD and βCD in H_2O at 25°C [a]

Complex	$-\Delta H^0$ (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})	$-\Delta G^0$ (kJ mol^{-1})
MR^-/αCD	20.9±1.2	6.1±3.9	22.7±2.4
MR^-/βCD	19.6±1.0	3.1±3.4	20.5±2.0
H_2MR^+/αCD	27.3±0.3	−22.5±1.1	20.6±0.6
H_2MR^+/βCD	18.9±0.2	3.7±0.8	20.0±0.4

[a] The uncertainties in ΔH^0 and ΔS^0 are the error estimates of the slope and intercept of equation *8*. The uncertainties in ΔG^0 were estimated according to the relation $\Delta G^0 = \Delta H^0 - 298\ \Delta S^0$.

$MR^-/\beta CD$) and the cation ($H_2MR^+/\alpha CD$ and $H_2MR^+/\beta CD$) of *p*-Methyl Red at 25°C. Based on the values of ΔG^0, the most stable inclusion complex is that of the anion with αCD; the other inclusion complexes are of comparable stability. All the inclusion processes of *p*-Methyl Red with αCD and βCD are exothermic and the magnitude of ΔH^0 is indicative of the involvement of weak interactions in the binding processes. The inclusion complex of the cation with αCD is entropy-destabilized. A similar result was obtained for the binding of the acid form of Methyl Orange with αCD[21]. Since several steps can be envisaged for the inclusion process of a cyclodextrin[6], it is difficult to comment quantitatively on the values of the thermodynamic parameters as reported in the present study.

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