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KINETIC STUDIES OF AZO DYE–α-CYCLODEXTRIN COMPLEXES. EVIDENCE FOR AN ISOMERIZATION STEP

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ABSTRACT

The kinetics of complex formation of a series of azo dyes with α-cyclodextrin (cyclomaltohexaose, α-CD) were examined by means of the temperature-jump method at pH 7.2, an ionic strength of 0.15M, and a temperature rise to 25°. All dyes were azobenzenes with a hydroxy group at C-4′ of one ring and a carboxy group at C-4 of the other ring. The data obtained with the dyes containing a substituent at C-2′ were interpreted as indicating an isomerization step coupled to the association of the dyes with α-CD.

INTRODUCTION

Inclusion complexes of cyclodextrins (cycloamyloses), especially those of α-cyclodextrin (cyclomaltohexaose, α-CD), have been extensively studied in recent years. Several books and articles have reviewed the structures and properties of cyclodextrins as well as different aspects of their inclusion complexes[1–3]. Most studies, however, are thermodynamic and directed to the measurement of binding constants. The number of kinetic studies reported is relatively small, even though they may yield information (especially related to the mechanism of complex formation) that is not directly obtainable from thermodynamic studies. Although experimental work and recent theoretical studies[4,5] have added a great deal to the understanding of the nature of the cyclodextrin inclusion-complexes, the actual mechanistic picture of the complexation process is still far from being complete. For example, a change in the conformation of the α-CD molecule upon the formation of an inclusion complex has been proposed[6,7], but no direct experimental evidence to support this proposal has been reported.

A kinetic investigation of various azo dye–α-CD complexes has been published earlier by this laboratory[8]. We now report further data on the kinetics of the azo dye–α-CD complex formation indicating the presence of an isomerization step associated with the process of complex formation.

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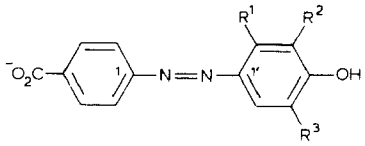

1 $R^1 = R^2 = R^3 = H$
2 $R^1 = H, R^2 = R^3 = Cl$
3 $R^1 = H, R^2 = R^3 = Me$
4 $R^1 = Me, R^2 = R^3 = H$
5 $R^1 = R^3 = Me, R^2 = H$
6 $R^1 = Me, R^2 = H, R^3 = NO_2$
7 $R^1 = Et, R^2 = R^3 = H$

EXPERIMENTAL

All the dyes used in this study (**1–7**) had been synthesized and purified during a previous investigation as described elsewhere[9]. They were used without further purification.

The kinetic data reported here were obtained by use of the temperature-jump method. The experiments were carried out using an instrument constructed in this laboratory[10] and previously employed in a study similar to the one described here[8]. Solution initially at 19.0 ±0.1° were heated by means of a 9-kV discharge which raised the temperature to 25°. The change in light intensity following a temperature jump was recorded with a Tektronix 564 storage oscilloscope.

The solutions used in temperature-jump experiments were prepared in phosphate buffer at pH 7.2 and an ionic strength of 0.15M. Total dye concentration in each solution was low enough so that the amount of dimerized dye, calculated by use of the reported dimerization constants[9], was less than ~10% of the total.

α-Cyclodextrin was purchased from Aldrich Chemicals Co. and purified as described previously[9].

RESULTS AND DISCUSSION

Within the time limits of the temperature–jump apparatus, each dye–α-CD solution gave a single relaxation*. In the case of the dyes **2** and **3**, the reciprocal relaxation times were plotted against the sum of the equilibrium concentrations of dye and α-CD, which were calculated by use of the previously reported association constants[9]. For the other dyes, α-CD was present in large excess and the reciprocal relaxation times were plotted against the total α-CD concentration.

*Very fast (average 49 ±8 μsec) and concentration independent relaxations were observed in the solutions of dye **1**–α-CD. Subsequent experiments showed that these relaxations were also present in the absence of α-CD. An analysis of the data suggested[11] that these relaxations were possibly due to an isomerization equilibrium of dye **1**. No additional relaxations were obtained when α-CD was present. With the other dyes, relaxations could be obtained only when α-CD was present

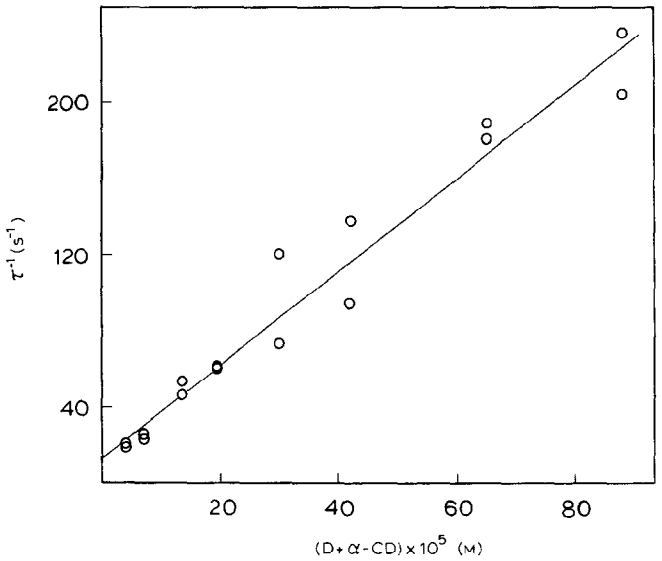

Fig. 1. Reciprocal relaxation time for the **3**–α-CD system plotted against the sum of the equilibrium concentrations of dye **3** and α-CD. Concentration of **3** for the extreme lower left data set at 37μM, for the rest of the data at 74μM. The line was drawn with a linear-least-squares method.

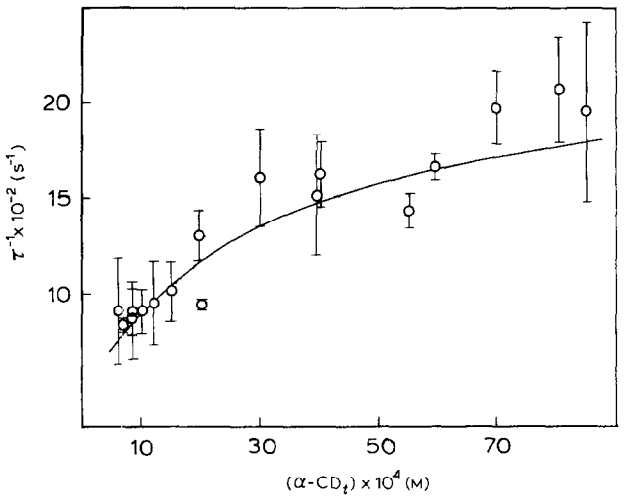

Fig. 2. Reciprocal relaxation time for the **4**–α-CD system plotted against total α-CD concentration. Concentration of **4**, 78μM. Results shown are combined data from two separate experiments. Each point is the average of two to three determinations and the error bars designate the standard deviations. The curve through the data points was drawn by use of the method explained in the text.

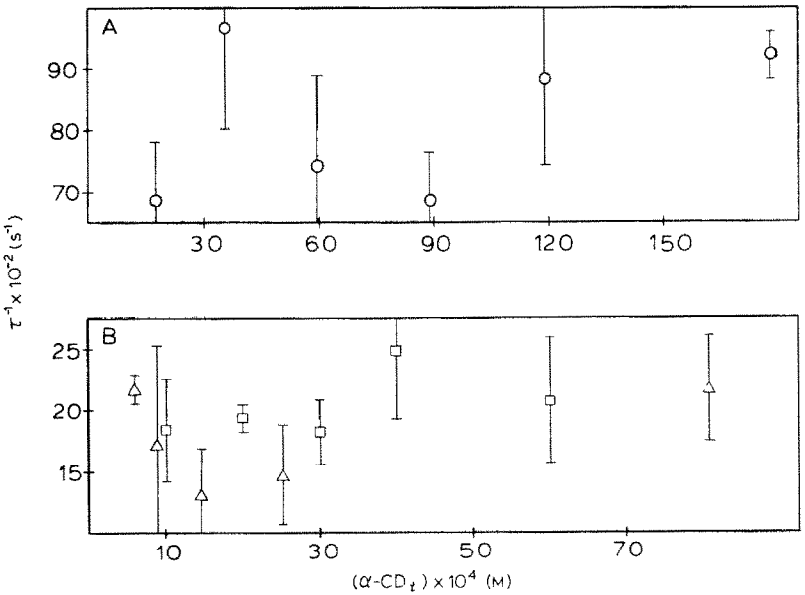

Fig. 3. Reciprocal relaxation times for solutions containing α-CD and one of the following dyes: (○) **7** (△) **5** and (□) **6**. The x-axes represent the total α-CD concentration. Dye concentrations: **5**, 33 μM; **6**, 19 μM; and **7**, 48μM Each poaint is the average of two to five determinations. The error bars designate the standard deviations.

Concentration dependencies of relaxation times exhibited three different types of behavior which enabled us to divide the dyes into three groups. In group I (**2** and **3**), the reciprocal relaxation times were found to be linearly related to concentration. A plot of a set of data obtained with the **3**–α-CD system is shown in Fig. 1. In group II (**4**), the reciprocal relaxation times increased with increasing α-CD concentration at low α-CD concentrations, but became independent of concentration at high α-CD levels (Fig. 2). Finally, in group III (**5–7**) within the limits of experimental error, the relaxation times were found to be independent of α-CD concentration, throughout the concentration range studied (Fig. 3).

In most cases, for purposes of comparison, the amplitudes of relaxations were also measured (in mm) and plotted against total α-CD concentration. In group I, amplitudes rose rapidly with increasing α-CD concentration, reached a maximum, and then gradually decreased with further increases in α-CD concentration. With the other dyes, the amplitudes rose with increasing α-CD concentration, then leveled off at high concentrations, and within the concentration range studied they did not fall back to smaller values.

In the case of the dyes in group I, the relaxation data implied a complexation mechanism of the type *1*.

$$\mathrm{D} + \alpha\text{-CD} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}\text{–}\alpha\text{-CD} \qquad (1)$$

The relaxation equation for this mechanism is given by Eq. (*2*)

$$\frac{1}{\tau} = k_1(\mathrm{D} + \alpha\text{-CD}) + k_{-1} \qquad (2)$$

where D stands for dye and D–α-CD for the complex of the dye with α-CD. Here in the species' symbols are also used to denote the equilibrium concentrations when needed. Concentration dependencies of the relaxation amplitudes obtained in group I also fit the type of behavior expected[12] from Eq. *1*. The rate constants for the complex formation of the dyes in group I with α-CD were, therefore, calculated from their respective plots of $1/\tau$ *vs.* (D + α-CD) by use of Eq. *2*. These rate constants are given in Table I.

TABLE I

RATE CONSTANTS FOR THE COMPLEXATION OF DYES **2** AND **3** WITH α-CYCLODEXTRIN[a]

Dye	$k_1\ (M^{-1}) \times 10^{-4}$	$k_{-1}\ (s^{-1})$
2	17.3 ±0.9	3.1 ±0.7
3[b]	19.1 ±1.8	23.8 ±4.0
	24.7 ±1.3	13.7 ±5.8

[a]Error limits given for the rate constants designate standard deviations. [b]Measurements with the **3**–α-CD system were performed twice on different dates.

The results presented in Table I must be treated with care however, since Eq. *1* may also arise as a specific case of a more general mechanism (Eq. *3*), as will be discussed later. In such a case, the rate constants, k_1 and k_{-1} of Eq. *2* may not be "true" rate constants, but they may instead be "apparent" constants composed of a collection of several rate constants[12].

In order to explain the relaxation data obtained with the **4**–α-CD system, the mechanism given in Eq. *3*, which includes two isomers of the complex, was employed. In Eq. *3* the subscripts of (D–α-CD) represent the two isomers of the complex.

$$\mathrm{D} + \alpha\text{-CD} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathrm{D}\text{–}\alpha\text{-CD})_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (\mathrm{D}\text{–}\alpha\text{-CD})_2 \qquad (3)$$

The equations for the two relaxation times associated with this mechanism were taken from the literature[12]. If the association step equilibrates much faster than the isomerization step, *i.e.*, $[k_1(\mathrm{D} + \alpha\text{-CD}) + k_{-1}] \gg [k_2, k_{-2}]$, the relaxation equations are given by Eqs. *4* and *5*

$$\frac{1}{\tau_F} = k_1(\mathrm{D} + \alpha\text{-CD}) + k_{-1} \tag{4}$$

$$\frac{1}{\tau_S} = k_2 \frac{K_1(\mathrm{D} + \alpha\text{-CD})}{1 + K_1(\mathrm{D} + \alpha\text{-CD})} + k_{-2} \tag{5}$$

where τ_F and τ_S stand for the fast and slow relaxation times corresponding to the faster association and the slower isomerization steps, respectively. In Eq. *5*, $K_1 = k_1/k_{-1} = (\mathrm{D}\text{–}\alpha\text{-CD})_1/(\mathrm{D})(\alpha\text{-CD})$ is the equilibrium constant for the association step. As mentioned earlier, in the case of the dyes in groups II and III, the concentration term in Eq. *5*, (D + α-CD), was assumed to be approximately the same as the total α-CD concentration, (α-CD_t). Eq. *5* predicts a curve that first rises with increasing α-CD concentrations, and then levels off and reaches a limit whose value is equal to ($k_2 + k_{-2}$). This kind of a curve clearly fits the relaxation data obtained with the **4**–α-CD system (Fig. 2).

To carry out a more detailed analysis of the data obtained with the **4**–α-CD system, Eq. *5* was rearranged into form *6* which

$$\frac{1}{1/\tau_S - k_{-2}} = \frac{1}{k_2} + \frac{1}{k_2 K_1(\alpha\text{-CD}_t)} \tag{6}$$

represents a straight line where $y = 1/(1/\tau_S - k_{-2})$ and $x = 1/(\alpha\text{-CD}_t)$. The intercept and slope are given by $1/k_2$ and $1/k_2K_1$, respectively. If k_2 and k_{-2} are known, the equilibrium constant of the isomerization step, $K_2 = k_2/k_{-2}$, can be calculated along with the overall association constant, $K_a = [(\mathrm{D}\text{–}\alpha\text{-CD})_1 + (\mathrm{D}\text{–}\alpha\text{-CD})_2]/(\mathrm{D})(\alpha\text{-CD}) = K_1 + K_1K_2$. K_a is assumed to represent the previously reported[9] association constants of the dye–α-CD complexes.

The unknowns in Eq. *6*, namely, k_2, k_{-2}, and K_1, were estimated with a trial and error process[13], *i.e.*, several values of k_{-2} were used to plot the relaxation data obtained with the **4**–α-CD system in the form of Eq. *6*. For each value of k_{-2} tried, k_2, K_1, K_2, K_a, and the standard deviation of the straight line were calculated. The value of k_{-2} that gave both a relatively small standard deviation and a K_a value close to the reported association constant[9] was chosen. Final results obtained in this

TABLE II

EQUILIBRIUM AND RATE CONSTANTS FOR THE COMPLEXATION OF DYE **4** WITH α-CYCLODEXTRIN

k_2	$= 1830\ \mathrm{s}^{-1}$
k_{-2}	$= 460\ \mathrm{s}^{-1}$
K_1	$= 310\ \mathrm{M}^{-1}$
K_2	$= 4$
$K_a{}^a$	$= 1550\ \mathrm{M}^{-1}$
$K_a{}^b$	$= 1590\ \mathrm{M}^{-1}$

[a]Calculated overall association constant. [b]Reported overall association constant[9].

fashion are given in Table II. The curve in Fig. 2 was drawn from Eq. *5* and the total α-CD concentrations along with the results given in Table II.

It is reasonable to assume that the relaxation times obtained with the dyes in group III in the presence of α-CD can also be represented by Eq. *5*. However, since the dyes in group III have all been shown[9] to bind to α-CD with association constants smaller than that of dye[4], the curvature in plots of $1/\tau_S$ *vs.* (α-CD$_t$) will become detectable only when the relaxation measurements are made over concentration ranges wider than those employed during this study. With the relaxation times available at the present, apparently concentration-independent, it was not possible to apply an analysis of the type carried out for the **4**–α-CD system to the dyes in group III.

The data and the arguments presented so far suggest that, for the dyes in groups II and III, there is an isomerization step coupled to the process of complex formation. On the other hand, such an isomerization step was not detectable during the complex formations of the dyes in group I. Dyes **4**–**7** have one common structural feature, namely, they all have a substituent group at C-2′, whereas in dyes **2** and **3** that position is unsubstituted. It is very likely that this basic structural difference between these dyes accounts for the kinetic differences observed. The isomerization step may be totally absent during the complex formation of the dyes **2** and **3** with α-CD, but may be induced whenever there is a substituent group at C-2′. If this is the case, then the rate constants obtained for the dyes **2** and **3** (Table I) will be the actual rate constants associated with Eq. *1*. On the other hand, a reaction of the type given by Eq. *1* may also arise as a specific case of Eq. *3*. Several pertinent examples are discussed by Bernasconi[12]. For example, if the isomerization step equilibrates faster than the association step, that is $[k_2 + k_{-2}] \gg [k_1(\mathrm{D} + \alpha\text{-CD}), k_{-1}]$, then the equation of the slower relaxation process will be identical to Eq. *2*, except for the fact that the second rate constant in it will now be composed of three of the four rate constants of Eq. *3*.

At the present, the available data are insufficient to make a choice between these cases and to reveal the exact nature of the isomerization step in Eq. *3*, including the precise role of the substituent group at C-2′. This step may arise as a result of the isomerization, for example tautomerization, of the dye molecules in the α-CD cavity. Another possibility involves the isomerization of α-CD, causing a guest molecule to move partially in and out of its cavity. It has been suggested that an "empty" α-CD molecule in water exists in a distorted conformation, which arises as a result of the rotation of one or more D-glucose rings around glycoside linkages[6,7], and that this distorted conformation is relieved when α-CD forms an inclusion complex[6,7]. In a similar fashion, the first step in Eq. *3* may represent the formation of a complex with the dye molecule only partially in the cavity of α-CD, and the subsequent isomerization step may be due to the change of the conformation of α-CD to better accommodate the dye molecule, resulting in a tighter complex. Previous data indicate that the dyes used in this study enter the α-CD cavity with the carboxylic end first[9]. It is, therefore, possible that a substituent at C-2′ will

interfere with the rotation of the D-glucose ring(s) and slow down the rate of isomerization. In the absence of such a group, *i.e.*, in **2** and **3**, the isomerization step may be too fast to be detected with the temperature-jump method. In which case, only the slower relaxation corresponding to the association step represented by a linear equation, as discussed in the preceding paragraph, will be observed. It should be pointed out that these discussions apply only to those dyes having an alkyl group at C-2′. Dyes containing other types of substituents in this position, *i.e.*, polar groups, etc., may give rise to complex-formation mechanisms different from the ones discussed here.

In summary, the data presented in this report indicate that the formation of α-CD inclusion complexes may not always be one-step processes as they have usually been assumed[8,14], but that in certain cases a more complex mechanism may be involved calling for more detailed and careful analyses.

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