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Note

The effect of dimethyl sulfoxide on the kinetics of azo dye–cyclomaltohexaose (α-cyclodextrin) inclusion complex formation

AYDIN ÖRSTAN*

Department of Biochemistry, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, New York 10029 (U.S.A.)

AND JOHN F. WOJCIK

Department of Chemistry, Villanova University, Villanova, Pennsylvania 19085 (U.S.A.)

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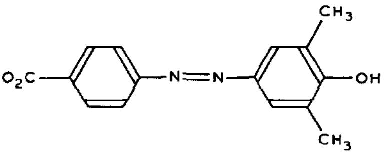
Thermodynamic studies indicate that the inclusion complexes of cycloamyloses (cyclodextrins) are less stable either in pure organic solvents[1], or in water–organic solvent mixtures[2–4], than they are in pure water. Furthermore, according to the results of Harrison and Eftink[2], and Örstan and Ross[4], the macroscopic solvent surface tension appears to be a critical solvent property in determining the thermodynamic stabilities of cycloamylose complexes.

To investigate the solvent effects on the kinetics of the formation of cycloamylose complexes, we have measured the rate constants for complex formation between cyclomaltohexaose (α-CD) and an azo dye (**1**) in mixtures of water with dimethyl sulfoxide (Me_2SO) at 25° by use of the temperature-jump method. Our previous results[5] indicated that the formation of a complex between dye **1** and α-CD in aqueous solution can be represented by Eq. (*1*), where k_1 and k_{-1} are the forward



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*To whom correspondence should be addressed at: Institute of Gerontology, University of Michigan, 300 N. Ingalls, Ann Arbor, Michigan 48109-2007 (U.S.A.).

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$$\alpha\text{-CD} + \mathbf{1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{1}\text{-}\alpha\text{-CD} \tag{1}$$

and reverse rate constants, respectively. We have assumed that the mechanism given in Eq. (*1*) is also valid in water–Me_2SO, provided that the formation of a complex between Me_2SO and α-CD is incorporated into this scheme (Eq. *2*), where

$$\alpha\text{-CD} + \mathbf{1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{1}\text{-}\alpha\text{-CD} \qquad \alpha\text{-CD} + Me_2SO \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Me_2SO\text{-}\alpha\text{-CD} \tag{2}$$

k_2 and k_{-2} are the forward and reverse rate constants, respectively, for the formation of the Me_2SO–α-CD complex. Thermodynamic evidence for the formation of a Me_2SO–α-CD complex in aqueous solution has been given by Gelb *et al.*[6]. The equilibria in Eq. (*2*) can be represented by the following association constants (Eqs. *3* and *4*). Here and in the following equations, the symbols in parentheses represent

$$K_1 = (\mathbf{1}\text{-}\alpha\text{-CD})/(\alpha\text{-CD})\,(\mathbf{1}) \tag{3}$$

$$K_2 = (Me_2SO\text{-}\alpha\text{-CD})/(\alpha\text{-CD})\,(Me_2SO) \tag{4}$$

the molar equilibrium concentrations. Since the molecule of Me_2SO is smaller than that of dye **1**, the Me_2SO-binding step can be assumed to equilibrate much faster than the dye-binding step. This assumption is supported by the observation that the forward rate constant for the binding of butanol (the molecule of which is about the size of that of Me_2SO) to α-CD is approximately 2×10^3-fold larger[7] than that for the binding of the azo dye **1** to α-CD. Under this condition, the reciprocal of the relaxation time for the slower dye-binding step[8] is given by Eq. (*5*). Since the total

$$\frac{1}{\tau} = k_1 \left\{ (\alpha\text{-CD}) + (\mathbf{1}) \left[\frac{1 + K_2\,(\alpha\text{-CD})}{1 + K_2\,[(\alpha\text{-CD}) + (Me_2SO)]} \right] \right\} + k_{-1} \tag{5}$$

Me_2SO concentrations, (Me_2SO_t), were much higher than the total α-CD concentrations, we have assumed that $(Me_2SO) \cong (Me_2SO_t)$.

The rate constants at each Me_2SO concentration were calculated as follows. First, the concentration of Me_2SO, originally expressed as volume percent, was converted to mol/L by use of the density data[9] for water–Me_2SO mixtures. Then, by use of the apparent values[3] of K_1, along with Eqs. (*3* and *4*) and the mass conservation relations for each species, the equilibrium concentrations of α-CD and **1** were calculated*. Subsequently, the rate constants, k_1 and k_{-1}, were obtained

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*According to Gelb *et al.*[6], in water containing 10 and 20% Me_2SO, the values of K_2 at 25° are 0.41 and $0.37\,\text{M}^{-1}$, respectively. For our calculations we used the average (0.39) of these values.

from a linear plot drawn according to Eq. (5). By use of the new value of K_1 obtained from the ratio of the rate constants, the calculations were repeated until the difference between the initial and final values of K_1 during an iterative step were within 1% of each other.

The rate constants obtained with this procedure are given in Table I. These results indicate that as the Me_2SO concentration is increased, the value of k_1 decreases but, within experimental error, the value of k_{-1} remains unhanged. Also included in Table I are the values of K_1 obtained from the ratio of the rate constants. Thermodynamically measured values of K_1 are given in the last column of Table I. These were obtained by correcting the apparent **1**–α-CD association constants[3], (K_a), for Me_2SO binding by use of the relationship, $K_1 = K_a\,[1 + K_2(Me_2SO)]$.

Since the pKa values of both phenol and benzoic acid in Me_2SO solution increase over the values of the water solutions[10,11], increasing concentrations of Me_2SO in water may also change the degree of ionization of the azo dye **1**, and consequently affect the rate constants. In water at 25°, the pKa values of the carboxyl and hydroxyl groups of **1** are 3.2 and 8.3, respectively[12]. In water at pH 7.2, the hydroxyl group is only ~7% ionized. Suppression of its ionization by Me_2SO will, therefore, have a negligible effect on the formation of the complex. Furthermore, when the volume fraction of Me_2SO in water–Me_2SO is 0.3 (~30%, v/v), the pKa value of benzoic acid is only ~0.5 unit higher than its value in pure water[11]. This suggests that in the water–Me_2SO mixtures used in this study, the pKa value of the carboxyl group of **1** did not increase significantly either. In any case, if no other factors were involved, the suppression of the ionization of the carboxyl group would probably increase the thermodynamic stability of the complex, as it is known that unionized benzoic acid derivatives form more stable complexes with α-CD than do the ionized derivatives[13]. The association constants, however,

TABLE I

KINETIC AND THERMODYNAMIC DATA FOR COMPLEX FORMATION BETWEEN CYCLOMALTOHEXAOSE AND THE AZO DYE AT 25° IN WATER-DIMETHYL SULFOXIDE MIXTURES[a]

Me_2SO^b	k_1 ($M^{-1}s^{-1}$) × 10^{-4}	k_{-1} (s^{-1})	K_1^c (M^{-1} × 10^{-3})	K_1^d (M^{-1} × 10^{-3})
0[e]	19.1	24	8.0	22.3
	24.7	14	17.6	
5	21.0	20	10.5	3.9
10	12.0	31	3.9	4.1
20	6.8	26	2.6	1.4
30	5.0	22	2.3	1.1

[a] The values k_1 and k_{-1} were calculated by the iterative procedure explained in the text. In all solutions, the total dye **1** concentration was 74μM. α-CD concentrations varied between 18μM and 1.8mM. [b] Volume percent (v/v). [c] The value K_1 was obtained from the ratio of k_1 to k_{-1}. [d] Thermodynamically measured values of K_1 were obtained by correcting the values from Gerasimowicz and Wojcik[3] as explained in the text. [e] Values of k_1 and k_{-1} in 0% Me_2SO are from Örstan and Wojcik[5].

indicate that the stability of **1**-α-CD decreases as the Me_2SO concentration is increased (Table I). Therefore, these arguments indicate that the decrease of k_1 in the presence of Me_2SO (Table I) is not due to the changes in the degree of ionization of the azo dye **1**.

To analyze our data further, we assumed that the dye (**1**), α-CD, and **1**-α-CD are in equilibrium with a transition state, **1**-α-CD‡. This assumption allowed us to treat the association and dissociation of **1**-α-CD according to the theory of absolute reaction rates[14] (Eqs. **6** and **7**). A relationship[14] between the measured forward rate

$$\alpha\text{-CD} + \mathbf{1} \rightleftharpoons \mathbf{1}\text{-}\alpha\text{-CD}^\ddagger \rightarrow \mathbf{1}\text{-}\alpha\text{-CD} \tag{6}$$

$$\mathbf{1}\text{-}\alpha\text{-CD} \rightleftharpoons \mathbf{1}\text{-}\alpha\text{-CD}^\ddagger \rightarrow \alpha\text{-CD} + \mathbf{1} \tag{7}$$

constants and the standard free energy of activation, $\Delta G^\ddagger$, is given in Eq. (*8*), where

$$\ln k_1 = \ln\left(\frac{k_b T}{h}\right) - \frac{\Delta G^\ddagger}{RT} \tag{8}$$

T is the absolute temperature, R is the gas constant, and k_b and h are Boltzmann and Planck constants, respectively.

According to a theory developed by Sinanoğlu[15], the standard unitary free-energy change for a reaction that proceeds with a decrease in the total surface area exposed to the solvent is expected to be linearly related to the solvent surface tension. Although the strict application of Sinanoğlu's theory requires the correction of the macroscopic surface tension for molecular dimensions[16], approximately

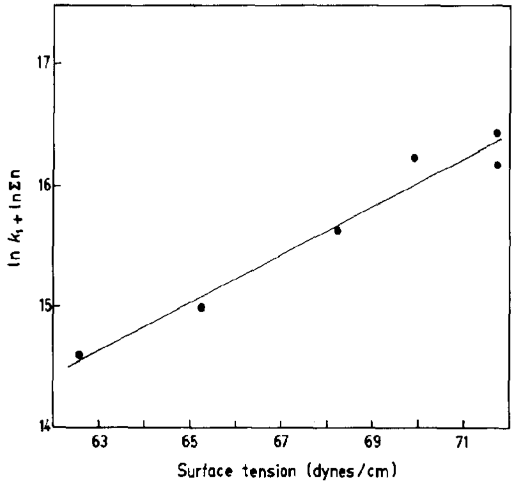

Fig. 1. Relationship of the natural logarithm of the forward rate constant to the surface tension of water–Me_2SO mixtures. The straight line was drawn from a linear least-squares analysis (the standard deviation and the correlation coefficient were 0.03 and 0.98, respectively).

linear relationships between standard free-energy changes and solvent macroscopic-surface tension in mixed solvents have been demonstrated for various molecular complexes[2,17]. For example, Harrison and Eftink[2] showed that a roughly linear relationship exists between surface tension and standard free-energy change for the formation of the complex of adamantanecarboxylate with cyclomaltoheptaose (β-cyclodextrin) in water–methanol mixtures. In addition, the results of Örstan and Ross[4] indicate that when the surface tension of water is decreased by addition of either ethanol or formamide, the stability of the complex of indole with cyclomaltoheptaose also decreases. The addition of calcium chloride, however, increased both the surface tension of water and the thermodynamic stability of the complex over that in pure water[4].

Similar arguments may be used to evaluate the effect of Me_2SO on k_1. If $\Delta G^{\ddagger}$ in Eq. (*8*) is linearly related to surface tension, then $\ln k_1$ will also depend linearly on surface tension[18]. As shown in Fig. 1, the plot of $(\ln k_1 + \ln \Sigma n)$ *vs.* surface tension is linear*. Therefore, using Sinanoğlu's arguments[15], we conclude that the formation of the transition state between α-CD and the dye molecule (Eq. *6*) is accompanied by a decrease in the total surface area exposed to the solvent. The apparent solvent independence of k_{-1} (Table I), however, indicated that the formation of the transiton state in Eq. (*7*) does not change the net surface area exposed to the solvent significantly, and that the transition between **1**–α-CD and **1**–α-CD‡ probably involves a conformational change within the complex. Thus, as far as the surface area exposed to the solvent is considered, the structures of **1**–α-CD and **1**–α-CD‡ are similar. These arguments also suggest that the decrease in the surface area exposed to the solvent during the formation of **1**–α-CD‡ in Eq. (*6*) is an important factor contributing to the thermodynamic stability of the complex.

EXPERIMENTAL

The details of the construction and the operation of the temperature-jump instrument have been published earlier[5,19,20]. Relaxation times were obtained from the oscilloscope tracings by the standard procedures[21], and the rate constants were calculated from the relaxation times as explained in the previous section. In the absence of α-CD, no relaxations were observed in dye **1** solutions.

The synthesis of the azo dye **1** and the purification of α-CD (Aldrich) have been described earlier[3]. Solutions containing **1** and α-CD were prepared in mixtures of Me_2SO with phosphate buffer (pH 7.2, *I* 0.15M). The percent concentration of Me_2SO was defined as the volume of Me_2SO relative to the final volume of the

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*Σn is the sum of the number of moles of water and Me_2SO in 1 L. This correction was applied to the rate constants because Sinanoğlu's derivations are based on unitary free-energy changes[15], *i.e.*, $\Delta G^{\ddagger}$ is calculated by use of an equilibrium constant expressed in mol fraction units. To calculate Σn, we used the densities of water–Me_2SO mixtures[9], and assumed that the contributions of the number of moles of buffer components, α-CD, and the dye **1** were negligible.

solution. The surface tensions of water–Me_2SO mixtures at 25° were obtained by interpolating the literature data[9].

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