December, 1975] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48(12), 3645—3647 (1975) 3645

The Retardation of the Benzidine Rearrangement of Hydrazobenzene by Cyclodextrins

Yoshihisa Matsui, Kazuo Mochida, Osamu Fukumoto, and Yoshio Date

Department of Agricultural Chemistry, Shimane University, Nishikawazu, Matsue 690

(Received March 7, 1975)

The benzidine rearrangement of hydrazobenzene is retarded by the addition of α-, β-, and γ-cyclodextrins. The effects on the rate of the cyclodextrins are decreased by the addition of cyclohexanol. The deceleration of the reaction by the cyclodextrins was attributed to the equilibrium complexation of the substrate in the cavities of the cyclodextrins, which may either restrict the large conformational change of the substrate necessary for reaching a sandwich-type transition state or lower the concentration of the diprotonated hydrazobenzene by means of the space alkalinity of cyclodextrins.

Cyclodextrins form monomolecular inclusion complexes with a variety of substrates in aqueous solutions and affect the reactivity of the substrates. Griffiths and Bender[1)] have explained the catalytic actions of cyclodextrins in terms of the nucleophilic catalysis, the microsolvent effect, and/or the conformational effect. However, there are relatively few examples in which the accelerations or decelerations of reactions are derived from the conformational effect of cyclodextrins. The present study will deal with the effects of cyclodextrins on the benzidine rearrangement of hydrazobenzene. It has been proven that the rearrangement requires a large conformational change to reach the transition state.

Experimental

Materials. The α-, β-, and γ-cyclodextrins were prepared by the method of Lane and Pirt.[2)] They were separated and purified according to the directions of Cramer and Henglein.[3)] The hydrazobenzene was prepared by the reduction of nitrobenzene with zinc dust and alkali in the presence of ethanol.[4)] Mp 125.5—127.0 °C (lit, mp 125 °C[4)]). Bindschedler's Green, used as an analytical reagent in following the rate of the rearrangement of hydrazobenzene, was prepared by the directions of Shine, Snell, and Trisler.[5)] A solution of Bindschedler's Green was standardized against a standardized solution of titanium trichloride.[5)] Reagent-grade cyclohexanol, methanol, citric acid, potassium citrate, and potassium sulfate were used without further purification.

Procedures. The reaction medium, generally 25 ml of a citrate buffer with a pH of 2.94 and with an ionic strength of 0.50 M adjusted by the use of potassium sulfate, was thermostated and degassed with N_2. To the solution we then added 3 ml of a 0.5 mM solution of hydrazobenzene in 60% methanol. Aliquots (2.0 ml) were pipetted out at regular time intervals and added to 10-ml standardized solutions of Bindschedler's Green. The residual amounts of Bindschedler's Green were colorimetrically determined at 712 nm with a Hitachi spectrophotometer, Model 124. The error in the determination of the hydrazobenzene concentration up to 1.00×10^{-4} M was about 0.01×10^{-4} M. The addition of cyclodextrins had virtually no effect either on the molar absorbance of Bindschedler's Green at 712 nm or on the determination of the hydrazobenzene contained in the solution. Therefore, the observed rate for the reaction of hydrazobenzene in the presence of cyclodextrins corresponds to the sum of the rate for the free hydrazobenzene and that for the complexed hydrazobenzene.

Results and Discussion

Hammond and Shine[6)] have shown that the rate of the benzidine rearrangement of hydrazobenzene increases in proportion to the hydrazobenzene concentration and to the square of the hydrogen-ion concentration. Similar results were also obtained in the present study in both the absence and the presence of 8.97 mM β-cyclodextrin. Since the reaction rate is very sensitive to the pH of the medium, the effects of carbohydrates were examined at a constant pH. The first-order rate constants in the presence of methyl α-D-glucopyranoside or α-, β-, or γ-cyclodextrin are summarized in Table 1.

The reaction of hydrazobenzene was little influenced by the addition of methyl α-D-glucopyranoside, whereas it was markedly retarded by cyclodextrins, especially

Table 1. First-order rate constants for the benzidine rearrangement of hydrazobenzene in the presence of carbohydrates at 25 °C

Carbohydrate	(mM)	pH[a)]	$k_{\text{obsd}}\times10^2\,\text{min}^{-1}$	$k_{\text{obsd}}/k_{\text{un}}$[b)]
None		2.94	1.51±0.02	1.00
Methyl α-D-gulco-pyranoside	62.5	2.94	1.60±0.02	1.06
α-Cyclodextrin	8.97	2.94	1.19±0.02	0.79
	17.9	2.94	1.01±0.01	0.67
	26.8	2.94	0.89±0.01	0.59
	35.9	2.94	0.74±0.02	0.49
	44.6	2.94	0.69±0.03	0.46
β-Cyclodextrin	0.90	2.94	1.04±0.01	0.69
	1.79	2.94	0.83±0.01	0.55
	3.59	2.94	0.56±0.02	0.37
	5.38	2.94	0.43±0.02	0.28
	7.18	2.94	0.32±0.02	0.21
	8.97	2.94	0.29±0.02	0.19
γ-Cyclodextrin	0.00	2.91	2.03±0.02	1.00
	4.59	2.91	1.63±0.02	0.80
	9.30	2.91	1.37±0.03	0.67
	13.6	2.91	1.16±0.01	0.57

a) Citrate buffer with the ionic strength of 0.50 M.
b) k_{un}: The first-orde rater constants in the absence of the carbohydrates.

TABLE 2. EFFECT OF CYCLOHEXANOL ON THE RATE OF THE REARRANGEMENT OF HYDRAZOBENZENE IN THE PRESENCE OF 8.97 mM β-CYCLODEXTRIN AT 25 °C AND pH 2.91

[Cyclohexanol], mM	$k_{obsd}\times10^2$, min^{-1}	k_{obsd}/k_{un}[a]
0	0.46 ± 0.01	0.23
11	0.75 ± 0.03	0.37
21	0.95 ± 0.01	0.47
32	1.29 ± 0.03	0.64
53	1.60 ± 0.02	0.79
106	1.72 ± 0.01	0.85

a) $k_{un}=(2.03\pm0.02)\times10^{-2}$ min^{-1}.

by β-cyclodextrin. This fact suggests that the retardation of the reaction by cyclodextrins is not due to the action of the glucopyranose groups, but to the inclusion of the substrate in the cavities of cyclodextrins. This presumption was substantiated by the examination of the effect of cyclohexanol on the rate-constants in the presence of 8.97 mM β-cyclodextrin at pH 2.91 (Table 2). It has been known that cyclohexanol is included in the cyclodextrin cavity[7,8] and that it competitively retards the catalytic action of cyclodextrins on substrates. As anticipated, the effect of the cyclodextrin on the reaction rate decreased with an increase in the concentration of cyclohexanol.

In order to evaluate the dissociation constant (K_{diss}) and the rate constant (k_c) for the inclusion complex between cyclodextrin and hydrazobenzene, the effect of the cyclodextrin concentration on the rate constant was examined and analyzed in the standard way on the basis of the Michaelis-Menten kinetics.[9] On the assumption of the formation of the 1 : 1 cyclodextrin–hydrazobenzene complex, the observed first-order rate constant (k_{obsd}) may be represented by Eq. (1), if the concentration of the cyclodextrin is much higher than that of hydrazobenzene:

$$k_{obsd} = K_{diss}(k_{un}-k_{obsd})/[\mathrm{CD}]_0 + k_c \tag{1}$$

where $[\mathrm{CD}]_0$ is the total concentration of the cyclodextrin. Figure 1 shows the plot of k_{obsd} *vs.* $(k_{un}-k_{obsd})/[\mathrm{CD}]_0$ for the reaction in the presence of β-cyclodextrin. The plot was virtually linear with a slope ($=K_{diss}$) of $(2.0\pm0.2)\times10^{-3}$ M and with an intercept ($=k_c$) of $(0.02\pm0.03)\times10^{-2}$ min^{-1}.[10] Although it might be possible that hydrazobenzene combines with two cyclodextrin molecules in a manner much like methyl orange,[11] the linearity of the above plot suggests that such complex formation, if present, is negligible. Similar linear plots were obtained for the reactions in the presence of α- and γ-cyclodextrins. The K_{diss} and k_c values determined are summarized in Table 3. It is apparent that the benzidine rearrangement of hydrazobenzene is virtually inhibited by the formation of the inclusion complexes with the cyclodextrins, although the K_{diss} values for α-, β-, and γ-cyclodextrins, were significantly different from one another.

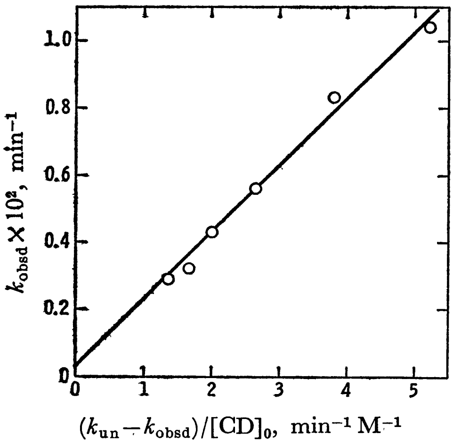
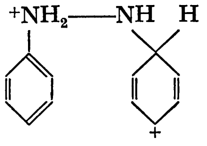


Fig. 1. The plot of k_{obsd} *vs.* $(k_{un}-k_{obsd})/[\mathrm{CD}]_0$ for the reaction in the presence of β-cyclodextrin.

TABLE 3. THE DISSOCIATION CONSTANT (K_{diss}) AND THE RATE CONSTANT (k_c) OF THE CYCLODEXTRIN-HYDRAZOBENZENE COMPLEX AT 25 °C

Cyclodextrin	pH	K_{diss}, mM	$k_c\times10^2$ min^{-1}
α	2.94	30 ± 8	0.15 ± 0.21
β	2.94	2.0 ± 0.2	0.02 ± 0.03
γ	2.91	20 ± 11	-0.10 ± 0.84

It has been proven that the rearrangement is intramolecular, proceeding through a sandwich-type transition state in which the two benzene rings lie over each other. Recently, Olah *et al.*[12] has suggested that the folded structure, **1**, is the most likely diprotonated intermediate.



1

When hydrazobenzene is included in the cavity of cyclodextrin, this large conformational change, accompanied by the rearrangement, may be suppressed by the rigid structure of the cyclodextrin. That is, the cyclodextrin may restrict the conformation of the diprotonated hydrazobenzene to the unfolded one. The importance of the conformational restrictions of substrates has often been reported in enzymatic reactions, too.

Another explanation, which is less probable, of the effect of the cyclodextrins is that they depress the protonation of hydrazobenzene. Cramer[13] has shown that the acid-catalyzed hydrolysis of indican is depressed by the formation of the inclusion complex with β-cyclodextrin. The author explained the effect of the cyclodextrin in terms of the "space alkalinity" of the cavity of the cyclodextrin. However, cyclodextrin generally acts as a weak acid[14] rather than as a base, and the concept of space alkalinity is itself rather doubtful. Thus, Broser and Lautsch[15] have shown that, although cyclodextrins do facilitate the acid dissociation of various indicator molecules, they do not actually act as proton acceptors, but, rather, act as electrophiles on the dissociated indicator molecules to stabilize them.

In order to examine the driving force for the interaction of hydrazobenzene with cyclodextrin, the

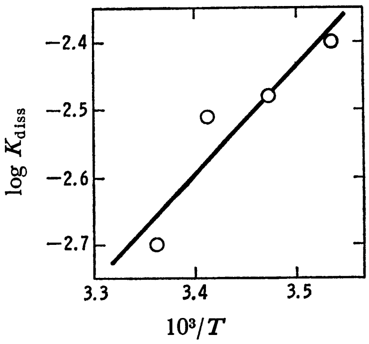


Fig. 2. The plot of log K_{diss} *vs.* the reciprocal of the temperature for the complex of hydrazobenzene with β-cyclodextrin.

thermodynamic parameters were determined for the complex formation. Figure 2 shows the plot of log K_{diss} *vs.* the reciprocal of the temperature for the complex of hydrazobenzene with β-cyclodextrin. The plot is approximately linear; the ΔH and ΔS for the dissociation of the complex were evaluated from the slope and the intercept to be -7.3 ± 6.0 kcal/mol and -36 ± 20 e.u. respectively.[10] It is evident that the driving force for the complex formation is entropic rather than enthalpic.

References

1) D. W. Griffiths and M. L. Bender, *Advan. Catal.*, **23**, 209 (1973).

2) A. G. Lane and S. J. Pirt, *J. Appl. Chem. Biotechnol.*, **21**, 330 (1971).

3) F. Cramer and F. M. Henglein, *Chem. Ber.*, **91**, 308 (1958).

4) W. J. Hickinbottom, "Reactions of Organic Compounds," 3rd Ed., Longmans, Green, London (1957), p. 462.

5) H. J. Shine, R. L. Snell, and J. C. Trisler, *Anal. Chem.*, **30**, 383 (1958).

6) G. S. Hammond and H. J. Shine, *J. Amer. Chem. Soc.*, **72**, 220 (1950).

7) R. Breslow and L. E. Overman, *ibid.*, **92**, 1075 (1970).

8) W. B. Gruhn and M. L. Bender, *Bioorg. Chem.*, **3**, 324 (1974).

9) G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942).

10) These values were obtained by the method of least-squares with the aid of a computer.

11) F. Cramer, W. Saenger, and H. -Ch. Spatz. *J. Amer. Chem. Soc.*, **89**, 14 (1967).

12) G. A. Olah, K. Dunne, D. P. Kelly, and Y. K. Mo, *ibid.*, **94**, 7438 (1972).

13) F. Cramer, *Ann. Chem.*, **579**, 17 (1953).

14) R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Amer. Chem. Soc.*, **89**, 3253 (1967).

15) W. Broser and W. Lautsch, *Z. Naturforsch.*, **B8**, 711 (1953).