Carbohydrate Research, 92 (1981) 177–181
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

A KINETIC INVESTIGATION OF THE BINDING OF AZO DYES TO CYCLOMALTOHEXAOSE

RONALD P. ROHRBACH* AND JOHN F. WOJCIK**
Department of Chemistry, Villanova University, Villanova, PA 19085 (U.S.A.)
(Received December 22nd, 1980; accepted for publication, January 17th, 1981)

ABSTRACT

The equilibrium constants and rate constants for the formation of azo dye–cyclomaltohexaose complexes have been determined at 26.6° and an ionic strength of 0.15. The effect of changing the substituents on the dyes is explained in terms of the sizes (steric factors) of the substituents and the charges on the substituents.

INTRODUCTION

The cyclomalto-oligosaccharides (cycloamyloses, or cyclodextrins), which are cyclic oligomers of D-glucose possessing a cavity of sufficient size to accept small molecules and form inclusion complexes, have been much studied in recent years from the viewpoints of both enzyme models[1] and practical applications[2]. They are themselves of interest, however, because the inclusion complexes formed by them involve, not the usual, covalent or electrostatic bonds found in most complexes, but nonspecific interactions in which the solvent plays a major role. Consequently, an understanding of the potential energy–reaction coordinate plot, and the nature of the transition state for the complexation reaction, would be most useful. Kinetic studies leading to such comprehension have been undertaken[3,4], in which rates of complexation of azo dyes and of small anions with these oligomers were investigated.

We now describe a further examination of the inclusion of azo dyes that leads to some insights into the features which need to be considered in any such study. Because the complexation reactions are fast, the temperature-jump method was utilized to examine the rate processes.

EXPERIMENTAL

All temperature-jump studies were conducted in a device, constructed in our laboratory[5], that was patterned after one in the laboratory of Prof. G. G. Hammes (Cornell University)[6]. The volume of the observation cell was ~0.4 cm^3. The light

*Current address: Universal Oil Products, Des Plaines, IL 60017, U.S.A.
**To whom correspondence should be addressed.

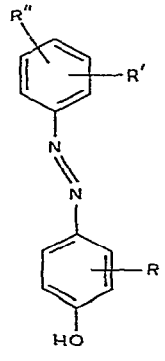
0008-6215/81/0000–0000/$ 02.50, © 1981 — Elsevier Scientific Publishing Company

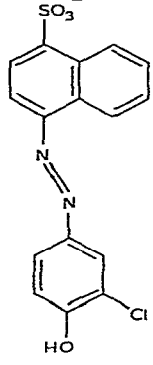
source was a 100-W, tungsten iodide lamp powered by a very low ripple-source (KEPCO JQE-10). Monochromatic light was provided by a Jarrell–Ash, quarter-meter grating monochrometer. After an initial stage of amplification by an emitter-follower circuit physically adjacent to the photomultiplier tube, further amplification was achieved, and a tracing of the change in light intensity was recorded, with a Tektronix 564 storage oscilloscope using a 2A9 differential amplifier. The ratio of the amplitude of the relaxation to the noise varied from 2:1 to 17:1. All circuitry employed in the Cornell T-jump apparatus was incorporated in ours.

The heating time was determined with a glycine buffer containing phenolphthalein, and was found to be ~ 5 μs for a discharge of 8 kV. The same chemical-system was used to obtain a plot of temperature change *vs.* discharge voltage. A 10-kV discharge (0.1 μF storage capacitor) gave a 7° change in temperature. The device was further tested by using the well studied, Co^{2+}–diglycine reaction[7], and it gave relaxation times that agreed with the literature values.

All of the dyes investigated in this study were azobenzene dyes (**1–13**), except for the phenylazonaphthalene dye **14**, and all had a hydroxyl group on C-4 of one phenyl ring, designated the unprimed ring.

The dyes were synthesized by standard, azo-coupling methods from readily





14

1 R = 3-Me, R' = 4-CHCHCOO$^-$, R'' = H
2 R = 3-Me, R' = 4-COO$^-$, R'' = H
3 R = 3-Me, R' = 3-COO$^-$, R'' = H
4 R = 3-Me, R' = 4-SO_3^-, R'' = H
(4-O$^-$), R' = 4-SO_3^-, R'' = H
5 R = H, (4 − O$^-$), R' = 4-SO_3^-, R'' = H
6 R = 3-Me, R' = 4-COO$^-$, R'' = 2-Me
7 R = 3-Me, R' = 4-COO$^-$, R'' = 3-OH
8 R = 2-Me, R' = 4-SO_3^-, R'' = H
9 R = 3-Et, R' = 4-SO_3^-, R'' = H
10 R = 3-$CHMe_2$, R' = 4-SO_3^-, R'' = H
11 R = 3-CMe_3, R' = 4-SO_3^-, R'' = H
12 R = 2,3-di-Me, R' = 4-SO_3, R'' = H
13 R = 3-Cl, R' = 4-SO_3^-, R'' = H

available phenols and anilines, and were recrystallized until a single spot was obtained in t.l.c. Analyses for C, H, and N, made by Galbraith Labs, were in agreement with the calculated values for the dyes containing carboxylic acid groups. However, although giving the correct C/N ratio, dyes having sulfonate groups had analyses that deviated somewhat from the calculated values. The total content of dye was determined by means of a $TiCl_3$ titration[8], and the content of water, by heating. By assuming that the dye was actually a mixture of pure dye, water, and salt (from the recrystallization procedure), all of the analytical data could be accounted for. Of the 14 dyes studied, nine (including all of the carboxylate dyes) had a purity of 95–100%, and that of the others was >86%. The actual percentage of dye present was accounted for in calculating the concentrations of solutions.

Dissociation constants for the dye complexes were determined spectrophotometrically with a Beckman DK-1 spectrophotometer. The spectrum of the dye was recorded at various concentrations of cyclomaltohexaose. If the dye gave evidence of being completely complexed by the oligomer, the molar absorptivity of the complexed form was obtained, and the equilibrium constant was then straightforwardly calculated. Otherwise (as in the case of the weaker complexes), the Benesi–Hildebrand method[9] was used. The solutions were maintained at pH values >5 and at an ionic strength of 0.15. Under these conditions, the carboxylic and the sulfonic groups are ionized. In two studies, the pH was maintained at >11, in order to ionize the phenolic hydroxyl group of the dye. (Our studies of dyes of similar structure have shown that dye aggregation is negligible at the concentrations used herein, and that the formation of higher complexes may also be neglected.)

RESULTS AND DISCUSSION

The values of the dissociation constants and the rate constants for the formation and dissociation of the complexes are given in Table I. All data refer to 26.6° and an ionic strength of 0.15. Because of the relatively small shift in the absorption spectrum on complexation of the dye, the experimental uncertainty in the equilibrium constants is ~30%, and that of the rate constants is ~50%. The rate constants are consistent with those obtained for similar dyes in an earlier study[3].

In order to interpret the results, it is necessary to decide which end of the dye enters the cavity of the cyclomaltohexaose. Two factors, namely, steric and charge, can prevent entry. As the oligomer is relatively rigid, the size of the cavity is fixed, and species that are too large will not enter the annulus. Thus, in the earlier work[3], it was discovered that a 4-hydroxy-3-methylphenyl group could enter the cavity, but a 4-hydroxy-3,5-dimethylphenyl group could not. The earlier work also showed that, on ionizing the hydroxyl group, the rate constants for entry decreased by a factor of $\sim 10^{-2}$.

With the earlier work in mind, our kinetic results may be interpreted as follows. Compounds **1–4**, **6**, and **7** enter with the 4-hydroxy-3-methylphenyl group first; this is supported by the facts that (*1*) the rate constants for the formation of these com-

TABLE I

DISSOCIATION CONSTANTS[a] AND RATE CONSTANTS[b] FOR THE FORMATION AND DISSOCIATION OF THE COMPLEXES

Dye	*pH*	K (M) × 10^5	k_f ($M^{-1}s^{-1}$) × 10^{-6}	k_r (s^{-1}) × 10^{-3}
1	5.34	6.5	3.4	0.24
2	5.30	7.0	2.1	0.20
3	5.34	17.0	0.79	0.20
4	5.40	11.0	0.57	0.13
	12.00	17.0	0.0055	0.0035
5	11.00	17.0	0.55	0.10
6	5.40	610.0	0.18	1.2
7	5.93	3.8	2.8	0.075
8	5.05	620.0	0.0015	0.014
9	5.25	13.0	0.033	0.0019
10	5.25	23.0	0.013	0.0028
11	5.25	61.0	0.0014	0.0022
12	5.25	560.0	0.0012	0.0085
13	5.25	4.7	1.2	0.16
14	5.25	180.0	0.70	3.5

[a] K is a dissociation constant. [b] k_f is the rate constant for the formation of the complex, and k_r is the corresponding constant for the dissociation of the complex. All data refer to 26.6° and an ionic strength of 0.15.

plexes are of the same order of magnitude as those for a similar entering-group in the earlier work, (*2*) the shift of the carboxylate group from C-4′ to C-3′ (compounds **2** and **3**) would be expected to have a larger effect on the rate constant for entry, as would the introduction of the 2′-methyl group in compound **6** and the 3′-hydroxyl group in compound **7**, and (*3*) ionization of the hydroxyl group in compound **4** changed the rate constant by a factor of $\sim 10^{-2}$, in agreement with the earlier work.

Compounds **8**, **11**, and **12** enter with the phenyl-4′-sulfonate end first; this is supported by the following considerations. Compounds **8** and **12** differ by a single methyl group (on C-3 of **12**). Were the end of the molecule bearing the 2-methyl group to enter the cavity, this change in steric requirements should be reflected in a change in the rate constant for entry, but, should the sulfonate end enter first, these molecules would behave identically, as the introduction of the 3-methyl group (onto **8** to afford **12**) occurs in a region that does not sterically interact with the oligomer. The kinetic parameters are, in fact, identical within experimental error. Similarly, compound **11** (having a 3-*tert*-butyl group) would probably be prevented from entering the cavity except by the sulfonate end, and, indeed, the rate constant for its formation is the same as that for **8** and **12**.

With these results as a guide, the remaining rate-constants may be interpreted. For instance, compound **5** could only be studied at a pH at which the phenolic hydroxyl group is ionized. Based on the foregoing concepts, the rate constant for the un-ionized form of compound **5** would be expected to be $\sim 5.5 \times 10^7$ $M^{-1}.s^{-1}$. On

considering the series **5** (un-ionized OH), **13**, (**14**), **4**, **9**, and **10**, in which the substituent on C-3 of the entering group is progressively increased in size from that of a hydrogen atom to that of an isopropyl group, the effect of increasing the bulkiness of the entering group may now be clearly seen. It should be noted that the rate constant for complex-formation in this series decreases as the size of the entering group increases.

If compounds **5** and **8** are representative of those for which a phenolate ion and a sulfonate ion, respectively, passes through the cavity, the 300-fold difference between the formation rate-constants for these groups is interesting. Although the sulfonate group is larger than the ionized oxygen atom of the phenolate group, it would not be expected to interact sterically with the cyclomaltohexaose to a significant extent. A reasonable explanation for the large difference lies in the difference in the solvation requirements of each group, as the phenolate ion would be expected to have its charge dispersed in the ring, whereas the sulfonate group will have its charge localized within the group itself.

A comparison may be made regarding the rate constants for the dissociation process. Compounds **2** and **6** differ only by the 2′-methyl group in **6**. Similarly **13** and **14** differ by the replacement of the 2′- and 3′-hydrogen atoms of **13** by the fused benzene ring, to give the bulky naphthalene ring. Although the formation constants are somewhat different within each of these pairs, the dissociation rate-constants are significantly increased by the introduction of the bulkier groups, suggesting that, on formation, after passing the maximum in the potential energy–reaction coordinate curve, those compounds having the bulkier groups are prevented from passing as far down the curve as those lacking the bulkier groups; consequently, the former have the higher rates of dissociation.

Finally, it may be noted that kinetic studies yield information that is not obtainable from equilibrium-constant measurements alone. For instance, ionization of the hydroxyl group in compound **4** changes the formation rate-constant by a factor of 100 while not changing the dissociation constant.

ACKNOWLEDGMENT

The principal author (J.F.W.) expresses his gratitude to Prof. G. G. Hammes for his hospitality during a sabbatical leave in the Summer of 1973.

REFERENCES

1 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978, 96 pp.
2 W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 344–362.
3 F. Cramer, W. Saenger, and H.-C. Spatz, *J. Am. Chem. Soc.*, 89 (1967) 14–20.
4 R. P. Rohrbach, L. J. Rodriguez, E. M. Eyring, and J. F. Wojcik, *J. Phys. Chem.*, 81 (1977) 944–948.
5 R. P. Rohrbach, Ph.D. Thesis, Villanova University, Villanova, PA, 1975.
6 E. J. Faeder, Ph.D. Thesis, Cornell University, Ithaca, NY, 1970.
7 G. G. Hammes and J. I. Steinfeld, *J. Am. Chem. Soc.*, 84 (1962) 4639–4643.
8 E. Knecht, E. Rawson, and R. Loewenthal, *A Manual of Dyeing*, Vol. II, Griffin, London, 1916, p. 843.
9 H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 71 (1949) 2703–2707.