determination of the energy gap between two emitting levels, the method of principal component decomposition used here has several additional features: (i) In the case of two sublevels, within certain limits we determine the emission spectrum of each sublevel. (ii) Because we work with the entire spectrum, we also determine the ratio of the radiative decay rates (eq 13 and Appendix), which was not obtainable by the Crosby method that uses just two wavelengths. (iii) Finally, the method of principal component decomposition provides a simple procedure for deciding the number of independent sublevel spectra. Thus the method applied here for analyzing the temperature dependence of triplet sublevel emission spectra should be more generally applicable to such problems, which occur in a variety of molecular systems.

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Appendix. Mathematical Addenda

(1) That the points (α_i, β_i) should lie on a line can be seen by summing over the entries in the vectors in eq 5. We have

$$\sum_j v_{ij} = 1 \qquad \sum_j v_j^{\mathrm{A}} = A \qquad \sum_j v_j^{\mathrm{B}} = B \tag{18}$$

Hence, from eq 5,

$$1 = A\alpha_i + B\beta_i \tag{19}$$

Use of the same procedure on eq 7 and 8 shows that the points $(\alpha^{(0)}, \beta^{(0)})$ and $(\alpha^{(1)}, \beta^{(1)})$ must lie on the extensions of the same line.

(2) Because the emission spectra were uncorrected for the wavelength dependence of the detector sensitivity, eq 13 needs a correction. The emission matrix v_{ij} can be expressed as

$$v_{ij} = \sum_{q=1}^{s} k_{qr} D_j f_j^{(q)} N_q(T_i) \Big[\sum_j \sum_{q=1}^{x} k_{qr} D_j f_j^{(q)} N_q(T_i)\Big]^{-1} \tag{20}$$

where k_{qr} is the radiative emission rate of level q, $f_j^{(q)}$ is the normalized emission spectra of sublevel q expressed as photons per unit wavelength, D_j is the detector sensitivity at wavelength λ_j, and $N_q(T_i)$ is the number of molecules in sublevel q at temperature T_i. The normalized uncorrected sublevel emission spectra are then defined as

$$y_j^{(q)} = D_j f_j^{(q)} \Big[\sum_j D_j f_j^{(q)}\Big]^{-1} \tag{21}$$

where the normalizing factors

$$u_q \equiv \sum_j D_j f_j^{(q)} \tag{22}$$

will not, in general, be identical for all sublevels q. Substitution of eq 21 and 22 into eq 20 yields

$$v_{ij} = \sum_{q=1}^{s} u_q k_{qr} y_j^{(q)} N_q(T_i) \Big[\sum_{q=1}^{s} u_q k_{qr} N_q(T_i)\Big]^{-1} \tag{23}$$

The fractional contribution of level q to the observed spectrum at temperature T_i then becomes

$$x_i^{(q)} = u_q k_{qr} N_q(T_i) \Big[\sum_{q=1}^{s} u_q k_{qr} N_q(T_i)\Big]^{-1} \tag{24}$$

Equation 24 leads to a modified form of eq 13

$$x_i^{(0)}/x_i^{(1)} = (2u_1 \bar{k}_{1r}/u_3 k_{3r}) e^{\Delta/kT_i} \tag{13'}$$

Since the emission spectra are in the same wavelength region, we expect that the correction factor $u_1/u_3 \sim 1$.

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Cyclohexaamylose Complexation with Organic Solvent Molecules

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Abstract: Weak inclusion complexes have been discovered between cyclohexaamylose (α-cyclodextrin) and several organic species (ethanol, 2-propanol, 2-methyl-2-propanol, cyclohexanol, dioxane, dimethyl sulfoxide and phenol) in aqueous solution. Formation constants for these complexes were determined by measuring the effect of the complexation on the pH of a cyclohexaamylose/acid/base buffer equilibrium. Enthalpies and entropies of complexation are calculated from the temperature dependences of the formation constants. The existences of true complexes were verified by (1) observing that these thermodynamic parameters correlate in the same manner as has been shown for other cyclohexaamylose complexes and (2) noting the similarities of ^{13}C NMR resonance behavior to that of well-established complexes.

In recent years there have appeared many reports of complexes of cycloamyloses and their derivatives with a wide variety of substrate species. Of the several experimental methodologies employed in these studies, those based on pH potentiometry have been particularly successful in terms of accuracy and convenience. In this laboratory we have used pH potentiometry to measure cycloamylose complexation constants of numerous aqueous organic acids, phenols and anions.[1-3] Other workers[4,5] have also utilized pH potentiometry similarly. Although the methodology has been

(1) Gelb, R. I.; Schwartz, L. M.; Johnson, R. F.; Laufer, D. A. *J. Am. Chem. Soc.* **1979**, *101*, 1869–1874.
(2) Gelb, R. I.; Schwartz, L. M.; Laufer, D. A. *Bioorg. Chem.* **1980**, *9*, 450–461.

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reported in detail in ref 1, a short recapitulation at this point provides a useful introduction to certain extensions to be described in this communication. The primary measurement is the pH of a solution of an aqueous acid–base conjugate pair. This pair will also be called the buffer components and will be denoted by HB and B^-. In a solution of known concentrations of HB and B^-, measurement of the pH together with estimates of activity coefficients yields a value for the acid dissociation constant of HB. If cycloamylose (which will be denoted by Cy) is introduced to the solution and complexes with either or both of the buffer components, the $HB/B^-/H^+$ equilibrium will be shifted and so a pH perturbation will be observed. The extent of perturbation depends on the difference in cycloamylose complex strength between the buffer components. If pH values are measured in a series of solutions containing varying proportions of buffer and cycloamylose and if activity coefficients can be estimated, it is usually possible to calculate complex formation constants for cycloamylose with both buffer components. Although this has been the essential methodology of the several reports mentioned above, we will here coin a new descriptive phase "buffer pH perturbation of the first kind" for reasons soon to be apparent. Correspondingly, the buffer components will be called "first-kind" substrates.

The method of the first kind is obviously limited to determining the complexation constant of cycloamylose with a particular acid/base species only in the presence of its conjugate. The conjugate must exist in sufficient concentration to establish the buffer system. However, there are many instances of interesting substrate species with no acid–base conjugate or with an inaccessible conjugate. For example, anions of strong acids and many water soluble organic species have no acid–base conjugate under ordinary conditions. Also it is common to find organic acids that cannot be made into suitable aqueous buffers because of limited solubility of the undissociated acid, whereas the anion forms complexes of interest with cycloamylose. One purpose of this communication is to demonstrate that cycloamylose complexation constants can be determined for this class of substrate by "buffer pH perturbation of the second kind". This means that if a "second-kind substrate" species S, which is a nonparticipant in an acid–base equilibrium but does interact with cycloamylose, is added to a solution containing both HB/B^- buffer and cycloamylose, the pH will be perturbed. The addition of S withdraws cycloamylose molecules from the previously established equilibria involving HB and/or B^- and this effect propagates to the primary $HB/B^-/H^+$ equilibrium. The extent of pH perturbation is directly related to the amount of S added and to the strength of the Cy·S complex which is thereby calculable.

This new methodology enables us to study cycloamylose complexation with an extensive array of substrates previously inaccessible for lack of a suitable experimental monitor of the extent of complexation. In this communication we will pursue two lines of investigation. Firstly, we seek to extend theories of bonding mechanisms to complexes with second-kind substrates featuring widely varying chemical and structural properties. This information may well lead to further insights into the nature of biological complexations in general. The second line is more specific and results from the fact that many previous studies of cycloamylose complexations and catalysis have employed experimental conditions in which substantial concentrations of supposedly indifferent electrolytes or organic solvents were present in reaction mixtures. Clearly, undetected interactions of cycloamylose with such solution components might play a significant role in experimental results and their interpretations.

Model Equations

In order to determine complexation parameters of interest in our cycloamylose–buffer–substrate solutions, we hypothesize a set of coupled nonlinear equations based on equilibria and conservation relationships. The equations and data treatment procedures for experiments involving only first-kind substrates were described in detail in ref 1. These are now extended to include second-kind substrates as well by simple modifications.

Our solutions are prepared with known analytical concentrations of buffer acid F_{HB}, buffer base F_{B^-} accompanied by an equivalent amount of alkali metal cation F_{M^+}, cycloamylose F_{Cy} which in this communication will be cyclohexaamylose, and a second-kind substrate F_S. We hypothesize that an equilibrated solution of these components will contain the uncomplexed species H^+, OH^-, HB, M^+, B^-, Cy, S, and the complexes CyHB, CyB^-, and CyS. Other conceivable complexes not listed are, for example, (1) ternary complexes Cy_2HB and Cy_2B^- which are eliminated by judiciously choosing a buffer system of benzoic acid/benzoate which forms no such ternaries,[1] (2) associations $S{\cdot}B^-$ and S·HB whose absences are confirmed by independent experiments of adding S to HB/B^- buffer and detecting no significant shift in pH, and (3) mixed CySHB and $CySB^-$ and ternary Cy_2S and CyS_2 complexes which are eliminated by inference during the data treatment procedure as will be explained later. The assumed equilibria are for the buffer

$$K_a = \frac{[H^+][B^-]}{[HB]} \frac{\gamma_{H^+}\gamma_{B^-}}{\gamma_{HB}} \tag{1}$$

for the complexes with first-kind substrates

$$K_{CyHB} = \frac{[CyHB]}{[Cy][HB]} \frac{\gamma_{CyHB}}{\gamma_{Cy}\gamma_{HB}} \tag{2}$$

and

$$K_{CyB^-} = \frac{[CyB^-]}{[Cy][B^-]} \frac{\gamma_{CyB^-}}{\gamma_{Cy}\gamma_{B^-}} \tag{3}$$

for complexes with a second-kind substrate

$$K_{CyS} = \frac{[CyS]}{[Cy][S]} \frac{\gamma_{CyS}}{\gamma_{Cy}\gamma_S} \tag{4}$$

the the water autoprotolysis equilibrium. Here bracketed species denote molar concentrations and subscripted γ quantities are molar activity coefficients. The relevant conservation equations are for each analytical component

$$F_{HB} + F_{B^-} = [HB] + [B^-] + [CyHB] + [CyB^-]$$

$$F_{Cy} = [Cy] + [CyHB] + [CyB^-] + [CyS]$$

and

$$F_S = [S] + [CyS]$$

and an electroneutrality balance which if S is electrically neutral is

$$[H^+] + F_{M^+} = [B^-] + [CyB^-] + [OH^-]$$

but is supplemented by [S] and [CyS] terms if S is charged.

The molar activity coefficients which appear in the equilibrium equations are provisionally taken as follows: those that refer to nonelectrolytes are assumed to be unity and those that refer to ions are calculated from the Debye–Hückel equation with temperature-dependent parameters from the tabulation of Robinson and Stokes[6] and with ion-size parameter values in nm of 0.90 for H^+, 0.35 for OH^-, and 0.70 for benzoate ions. The experimental pH vs. analytical concentration data were fitted to the model equations by an elaborate nonlinear regression computer program described elsewhere.[7] Here the primary parameter value to be extracted by this procedure is the complex formation constant K_{CyS}, but we generally solved for K_a as well since any inaccuracy in calibration of the pH meter propagates into an erroneous K_a value.

(3) Gelb, R. I.; Schwartz, L. M.; Cardelino, B.; Fuhrman, H. S.; Johnson, R. F.; Laufer, D. A. *J. Am. Chem. Soc.* **1981**, *103*, 1750–1757.
(4) Miyaji, T.; Kurono, Y.; Vekama, K.; Ikeda, K. *Chem. Pharm. Bull.* **1976**, *24*, 1155–1159.
(5) Connors, K. A.; Lipari, J. M. *J. Pharm. Sci.* **1976**, *65*, 379–383.
(6) Robinson, R. A.; Stokes, R. H. "Electrolyte Solutions", 2nd ed.; Butterworths: London, 1965.
(7) Schwartz, L. M.; Gelb, R. I. *Anal. Chem.* **1978**, *50*, 1571–1576.

If the known K_a value for benzoic acid were substituted as a fixed parameter, then any such inaccuracy would constitute an uncompensated determinate error and would preclude a satisfactory fit of the model equations to the experimental data. Previously measured values of the complexation constants of benzoic acid and benzoate ion with Cy[1] were substituted into eq 2 and 3 and held fixed.

Confirming and Exploratory Experiments

A trial experiment was done to confirm that buffer pH perturbation of the second kind would yield a value for a complex formation constant in agreement with one measured previously by a different technique. The test was done using Cy and *m*-nitrophenol whose complex had been determined[3] by buffer pH perturbation of the first kind. Although *m*-nitrophenol is acidic with $pK_a \cong 8.5$, in benzoic acid/benzoate buffer with pH in the range 3.8 to 4.5 the ratio of the phenol to the phenolate is about 10^4. There is virtually no phenolate present and so under these conditions the *m*-nitrophenol constitutes a second-kind substrate. An initial solution was prepared with 5 m*F* benzoic acid and 2 m*F* sodium benzoate to which alternately several small portions of solid Cy and solid *m*-nitrophenol were added. By this strategy pH values were recorded for solutions in which analytical concentrations of Cy and *m*-nitrophenol ranged up to 0.05 and 0.1 *F*, respectively, in a single series. The observed pH data were not used directly but were first corrected for the effect of aqueous Cy on electrode junction potentials using a simple correlation described in ref 1. Also, the molar concentrations of all species were corrected for the increasing volume of the solution due to additions of solid Cy and S. Partial molar volumes used for these minor corrections were determined by experiment or, in the case of some subsequent substrates, taken from handbook tabulations. This trial yielded a value of 111.±4 for K_{CyS} for *m*-nitrophenol at 25 °C and this is to be compared with the value 107 ± 5 found by linear interpolation between 20 and 30 °C values reported in ref 3 or with the value 117 calculated from $\Delta H°$ and $\Delta S°$ values reported in this same paper. The agreement is satisfactory. Furthermore, the root-mean-square fit corresponding to the weighted differences between the observed and calculated data was 0.005 pH and thus corresponded roughly to the precision of our measurements in this experiment. The pattern of residuals appeared to be random and this important observation implies that uncompensated determinate errors were not present. In other words, the experimental data were in concert with the model equations as hypothesized. If there had been significant concentrations of species such as CySHB, $CySB^-$, Cy_2S, or CyS_2, the omission of any such complex from the conservation equations would constitute a determinate error, and therefore a satisfactory fit of the data would not have been possible. Thus we conclude that the method of buffer pH perturbation of the second kind is a reliable means of determining complex formation constants at least under the conditions of the *m*-nitrophenol trial.

On this basis we proceeded to study complexes of acetonitrile and phenol with Cy and benzoic acid/benzoate. In each case we began by adding portions of the substrate to the buffer and detected no pH change. Thus we concluded that no interactions due to possible chemical reactions or to the presence of impurities occurred between the benzoate buffer and second-kind substrate samples. Our results are shown in Table I.

Weak Complexes of Organic Substrates with Cyclohexaamylose

It soon became apparent that many organic second-kind substrates form rather weak complexes with cycloamyloses. When a substrate forms a weak complex, a high concentration is required in order to effect a significant buffer pH perturbation. However, a high concentration of organic solute constitutes a mixed medium in which activity coefficients and electrode junction potentials must differ from values in aqueous media in which K_{CyHB} and K_{CyB^-} are measured. In order to study these weak complexes, we found it necessary to modify our technique. Rather than add successive portions of substrate to solutions of buffer and Cy, we begin with a solution of buffer (~1 m*F*) and an excess of organic species S and then add several sufficiently small portions of Cy so that the concentration of uncomplexed S remains essentially invariant during the experiment and thus the solvent characteristics of the mixed medium also remains invariant. It now becomes necessary to measure or to hypothesize mixed medium behavior of the several activity coefficients involved in the calculation. We begin by noting that the buffer pH as measured potentiometrically is related to the activity of hydronium ion which appears inplicitly in eq 1. To show this explicitly we rearrange that equation to the form

$$pK_a = pH + \log \frac{\gamma_{HB}}{\gamma_{B^-}} + \log \frac{[HB]}{[B^-]} \qquad (1a)$$

having substituted $pH = -\log \gamma_{H^+}[H^+]$. Any change in the activity coefficient of hydronium ion or in the γ_{HB}/γ_{B^-} ratio due to the presence of an invariant amount of S causes a fixed change in the first two terms on the right-hand side of eq 1a. Any change in electrode junction potential adds an unknown but fixed increment to the measured pH. We effectively transfer these unknown increments to the left-hand side of eq 1a by our practice of not prescribing the value of pK_a. Rather pK_a is treated as an unknown parameter. Its value is calculated in the regression analysis but is of no interest. Next we notice from eq 2, 3, and 4 that γ_{Cy} appears only together in a ratio with another activity coefficient involving a Cy complex. In eq 2, we see this ratio is $\gamma_{CyHB}/\gamma_{Cy}$ and in eq 4 it is γ_{CyS}/γ_{Cy}. Both ratios here involve nonelectrolyte molecules with similar structures. We would expect both HB and S to be included within the Cy cavity so that the complex would interact as a solute like an uncomplexed Cy molecule. Thus we hypothesize that each of these two ratios of nonelectrolytic activity coefficients is unity. The activity coefficients in eq 3 would present a difficult problem to estimate, but by fortunate circumstance this is not necessary. In ref 1 we reported that the strength of the Cy_{B^-} complex is much weaker than that of CyHB for benzoate–benzoic acid. This means that in a buffer of roughly equal proportions of HB/B^- only a small fraction of the benzoate ions are complexed. Thus K_{CyS} is quite insensitive to the CyB^- equilibrium of eq 3, and as a consequence the ionic activity coefficient ratio here is taken as unity and this can introduce negligible error. Finally we must consider the nonelectrolytic activity coefficients γ_S and γ_{HB}. γ_S could be determined by equilibrium vapor pressure measurements of aqueous solutions of S. Alternatively, we will for now limit our experiments to those substrates S whose Cy complexes can be determined using S concentrations within the Henry's law region. In this region nonelectrolyte solute activity coefficients γ_S are unity. For the buffer component benzoic acid, however, separate experiments must be done to determine γ_{HB} in each mixed medium that we employ. This is done by measuring the solubility of solid benzoic acid in each medium.

Before reporting the results of these solubility experiments, we remark that our several hypotheses about activity coefficient behavior in mixed medium can be accepted as valid only if the K_{CyS} values derived turn out to be invariant with S concentration. We proceeded to make buffer pH perturbation measurements using second-kind substrates ethanol, dioxane, and dimethyl sulfoxide (Me_2SO). In each case we found that if we assumed an invariant $\gamma_{HB} = 1$, that the resulting apparent K_{CyS} values were markedly dependent on the concentration of substrate S. For example, we calculated for ethanol values of 4.58 ± 0.04 and 1.67

Table I. Complex Formation Constants of Cyclohexaamylose

substrate	temp, °C	K_{CyS}[a]
acetonitrile	10	6.3 ± 0.2
	15	6.0 ± 0.2
	25	5.6 ± 0.1
	35	4.8 ± 0.1
	45	3.9 ± 0.1
phenol	15	23.2 ± 1.0

[a] Uncertainties are standard errors estimated from the nonlinear regression fit to the experimental data.

Table II. Solubilities and Activity Coefficients of Benzoic Acid in Several Mixed Media at 25 °C

second-kind substrate	S concn, v_S/v_{H_2O}, %	benzoic acid solubility,[a] F	molar act. coeff, γ_{HB}[b]
	0	0.0275	1.000
ethanol	4	0.0301	0.92
	10	0.0356	0.77
2-propanol	4	0.0305	0.91
2-methyl-2-propanol	4	0.0305	0.91
acetonitrile	4	0.0335	0.82
	10	0.0478	0.57
	25	0.125	0.212
dioxane	4	0.0354	0.77
	10	0.0518	0.53
dimethyl sulfoxide	10	0.042	0.64
	20	0.063	0.42

[a] Approximately ±1% uncertainty by replication. [b] Approximately ±2% uncertainty.

± 0.10 in 4% and 10%, v/v solutions, respectively; for dioxane, values of 6.44 ± 0.09 and 9.40 ± 0.36 in similar S concentrations; and for Me_2SO, values of 1.27 ± 0.04 in 10% and 1.67 ± 0.03 in 20% S solution. These variations clearly signalled an error in the methodology. The remedy was to correct for the varying activity coefficient of benzoic acid with S concentration.

We measured the solubility of benzoic acid in several aqueous solutions of second-kind organic substrate by repeatedly analyzing the supernatant liquid for dissolved acid by titration with standardized NaOH. Replicate experiments showed that this procedure yielded solubilities precise to 1%. We found that if the concentration of S in the mixed medium was 2% v_S/v_{H_2O}, the observed solubility of benzoic acid was the same as that in H_2O to within this 1% precision. Since we have assumed that $\gamma_{HB} = 1$ in purely aqueous solution, we extend this assumption to mixed medium solutions as well up to the 2% level. Higher concentrations of organic substrate significantly increase the solubility of benzoic acid as shown in Table II. At any fixed temperature the activity of undissociated benzoic acid in a saturated solution is fixed and has the numerical value of the molar concentration of undissociated acid [HB] in aqueous solution where we have taken $\gamma_{HB} = 1$. [HB] is calculated from the observed analytical concentration F_{HB} by subtracting the concentration of dissociated acid. Because this correction is very small (<5% of F_{HB}), it was sufficiently accurate for this purpose to estimate its value as $(K_aF_{HB})^{1/2}$ where K_a is the known dissociation constant of benzoic acid. Activity coefficients γ_{HB} are then calculated in each mixed medium as the ratio of aqueous [HB] to mixed medium [HB] and these results are shown in Table II. The uncertainties of these activity coefficients are about ±2% and are primarily due to the uncertainties in the solubility measurements. We repeated several of these experiments at 45 °C and found that the γ_{HB} values obtained were within 2% of the 25 °C values. Hence, we will assume that γ_{HB} is independent of temperature over the range of our experiments.

With these measured benzoic acid activity coefficients and with the hypotheses enumerated above, we recalculated values for the complexation constants of various organic substrates and report these results in Table III. The uncertainties quoted for these K_{CyS} values are from a propagation-of-variance calculation which combines the uncertainties in γ_{HB} with those of the pH measurements.

Discussion of Observed Complexation Constants

An examination of comparable K_{CyS} values reported in Tables I and III shows that good agreement is obtained by the two variations of the buffer pH perturbation methodology. The agreement can be seen by the systems Cy·phenol at 15 °C and Cy·acetonitrile at 25 °C. Furthermore, the concentration independence of K_{CyS} for dioxane and Me_2SO with 6-Cy serves to confirm the validities of the several hypotheses and the benzoic acid solubility results inherent in these K_{CyS} values. It is particularly significant that K_{CyS} is independent of S concentration

Table III. Complexation Constants of Cyclohexaamylose with Organic Substrates

substrate	temp, °C	S concn, v_S/v_{H_2O}, %	K_{CyS}[a]
ethanol	15	4	4.6 ± 0.2
	25	4	4.1 ± 0.2
	25	10	4.8 ± 0.3
	35	4	4.3 ± 0.2
	45	4	4.0 ± 0.2
2-propanol	15	4	5.1 ± 0.2
	25	4	4.6 ± 0.2
	35	4	4.8 ± 0.2
	45	4	4.7 ± 0.3
2-methyl-2-propanol	15	4	4.1 ± 0.2
	25	4	4.1 ± 0.2
	35	4	4.4 ± 0.2
	45	4	4.4 ± 0.2
cyclohexanol	15	1	77 ± 2
	25	1	62 ± 1
	35	1	56 ± 1
	45	1	46 ± 2
dioxane	15	4	5.3 ± 0.3
	25	4	4.4 ± 0.3
	25	10	4.5 ± 0.5
	35	4	4.0 ± 0.2
	45	4	3.6 ± 0.2
dimethyl sulfoxide	25	10	0.41 ± 0.04
	25	20	0.37 ± 0.04
phenol	15	1	25.9 ± 0.3
	25	1	20.7 ± 0.3
	35	2	19.8 ± 0.3
	45	2	15.3 ± 0.4

[a] Uncertainties are standard error estimates which reflect both benzoic acid solubilities and buffer pH perturbation experiments.

Table IV. Standard Enthalpies and Entropies of Cyclohexaamylose Complexation

substrate	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, cal mol^{-1} K^{-1}
acetonitrile	−2.4 ± 0.3	−4.7 ± 1.1
ethanol	−0.7 ± 0.4	0.6 ± 1.2
2-propanol	−0.4 ± 0.4	1.9 ± 1.1
2-methyl-2-propanol	0.5 ± 0.2	4.6 ± 0.6
cyclohexanol	−3.0 ± 0.3	−1.8 ± 0.9
dioxane	−2.3 ± 0.2	−4.6 ± 0.7
phenol	−3.0 ± 0.5	−3.8 ± 1.8

up to the level of 20% v_S/v_{H_2O} utilized. The concentration of H_2O at these levels is substantially less than the H_2O concentrations in media with lesser amounts of S, and yet the equilibrium constant K_{CyS} does not change. This implies that the bonding mechanism for the formation of complexes in these cases cannot involve water molecules in any significant way. This means that such suggested mechanisms as release of water from the Cy cavity[8] and hydrophobicity of Cy[9,10] cannot be important here.

Having measured K_{CyS} values as functions of temperature in several systems, we derived standard enthalpy and entropy changes for the complex formation reactions by calculating least-squares van't Hoff lines. These $\Delta H°$ and $\Delta S°$ results appear in Table IV. Not long ago we reported[3] a correlation between $\Delta H°$ and $\Delta S°$ of complexation of Cy with about 20 substrates of various molecular structures. That correlation is

$$\Delta H° = (403 \pm 24)\Delta S° - (1.2 \pm 0.4) \times 10^3 \quad (5)$$

where $\Delta H°$ is in cal mol^{-1} and $\Delta S°$ is in cal mol^{-1} K^{-1}. Each entry in Table IV is within experimental error of this same correlation, as can be seen by substituting the standard error limits of the tabular $\Delta S°$ values into eq 5 and confirming that predicted $\Delta H°$

(8) Van Etten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242–3253.
(9) Komiyama, M.; Bender, M. L. *J. Am.* Chem. *Soc.* **1978**, *100*, 2259–2260.
(10) Saenger, W. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344–362.

Table V. ^{13}C NMR Resonance Displacements of Cyclohexaamylose and Organic Substrates upon Complexation at 30 °C

substrate		carbon no. C1	C2	C3	C4	C5	C6	SE[a]
		Cy Resonance, δ_0^{Cyn}						
		102.41	72.88	74.47	82.26	73.06	61.59	0.02
		Cy Resonance Displacement, $\Delta\delta^{Cyn}$, ppm						
phenol		0.16	0.02	−0.01	0.11	0.07	−0.09	0.05
cyclohexanol		0.32	0.00	0.15	0.15	0.14	−0.12	0.05
acetonitrile		0.19	−0.04	0.32	−0.05	−0.02	−0.29	0.05
dioxane		0.23	0.01	0.14	0.14	0.14	−0.14	0.05
2-propanol		0.30	−0.04	0.06	0.16	0.13	−0.19	0.05
2-methyl-2-propanol		0.28	−0.07	−0.02	0.21	0.14	−0.14	0.06
ethanol		0.23	0.01	0.14	0.14	0.14	−0.14	0.05
dimethylsulfoxide		0.52	−0.10	0.26	0.55	0.26	−0.10	0.10
		Substrate Resonance and Displacement, ppm						
phenol	δ_0^{Sn}	156.57	116.51	131.04	121.66			0.01
	$\Delta\delta^{Sn}$	0.35	0.29	−0.29	−0.53			0.12
cyclohexanol	δ_0^{Sn}	71.43	35.40	24.88	25.99			0.02
	$\Delta\delta^{Sn}$	−0.34	0.62	0.78	0.60			0.07
acetonitrile	δ_0^{Sn}	120.32	1.91					0.01
	$\Delta\delta^{Sn}$	−1.42	−0.19					0.09
dioxane	δ_0^{S1}	67.62						0.02
	$\Delta\delta^{S1}$	0.40						0.29

[a] Standard error estimates are calculated from the multiple regression analysis assuming an uncertainty of ±0.02 ppm for each resonance measurement.

values are within the standard error limits of the tabular $\Delta H°$ values. We believe that this observation provides particularly important verification that the pH perturbations that we measure do, indeed, reflect the formation of complexes between Cy and the organic second-kind substrates we are studying. Without this quantitative evidence, it might not be possible to resolve the ambiguity of pH perturbation due to solvent effects and that due to complex formation.

In order to provide still further confirmation of complex formation, we undertook to measure ^{13}C NMR spectra of both Cy and the substrates. We have previously reported certain patterns of ^{13}C NMR chemical shift displacements due to Cy complexation,[3] and so we look for similar patterns as further substantiating evidence of complexation between Cy and organic second-kind substrates.

Aqueous solutions (5% D_2O v/v) containing second-kind substrates (0.06–1.1 *F*) and Cy (0.02–0.1 *F*) were scanned by ^{13}C NMR spectrometry. As reported in an earlier publication,[3] we extract intrinsic ^{13}C chemical shifts of free (δ_0^{Cyn} or δ_0^{Sn}) and complexed (δ_1^{Cyn} or δ_1^{Sn}) binary species from observed resonances (δ_{obsd}^{Cyn} or δ_{obsd}^{Sn}) and known analytical concentrations using model equations which express equilibrium and conservation relationships. In our notation, subscripts 0 and 1 refer to free and binary complexed species, respectively, whose nonequivalent carbons are indexed by the integer *n*. Complexation constants required in these calculations were obtained from buffer pH perturbation experiments as described earlier in this communication. All spectra reflected fast-exchange conditions; i.e., only six resonances corresponding to nonequivalent Cy carbons and the expected number of lines due to substrate species were detected above the noise level. In Table V we show assignments of ^{13}C resonances as based on published values[11] and complexation displacements of resonances of Cy ($\Delta\delta^{Cyn} \equiv \delta_1^{Cyn} - \delta_0^{Cyn}$) and of the substrates ($\Delta\delta^{Sn} \equiv \delta_1^{Sn} - \delta_0^{Sn}$). We note similarities of some displacements with previously reported complexes. The Cy displacements induced by acetonitrile are similar to those of acetic acid and *p*-cyanophenol,[3] suggesting insertion of the cyano group through the C2, C3 rim of the cavity. The strong shielding of the cyano functionality ($\Delta\delta^{S1} = -1.42$ ppm) is consistent with this mode of binding. Likewise, the similarity between the $\Delta\delta^{Cyn}$ values of phenol and benzoate ion[3] implies that the phenyl group is oriented C4-end first into the cavity. Cyclohexanol, on the other hand, perturbs the Cy carbons substantially less than hexanecarboxylic acid[3] and this may be ascribed to the absence of CO_2H–Cy interactions, particularly at C5 and C6, in the cyclohexanol complex. Perturbation of the cyclohexanol's carbons noted here indicates that this substrate is at least partially included in the Cy cavity.

It is noteworthy that in all cases except that of acetonitrile we observe significant displacements of the C1, C4, and C5 resonances of Cy. These carbons do not bear hydroxyl groups and thus would not likely be affected by interactions with the bulk solvent. Thus we conclude that these displacements are due to formation of inclusion complexes with the corresponding second-kind substrate. We notice that phenol, acetonitrile, and dioxane have displacements $\Delta\delta^{Cy1}$ at the C1 carbon of Cy and complexation $\Delta H°$ values which conform to the correlation reported in ref 3 but cyclohexanol, 2-propanol, ethanol and 2-methyl-2-propanol do not. The reasons for these discrepancies are not clear at this time.

From the foregoing studies we conclude that cyclohexaamylose forms weak inclusion complexes with small organic molecules. Among these the most notable is acetonitrile, which has been employed as a solvating agent in many experiments designed to monitor the catalytic efficiency of the cycloamyloses in ester hydrolyses reactions.[9,12] Being unaware of the acetonitrile complexation, these investigators could not have made the necessary adjustments in calculating catalytic rate enhancements so that these enhancements were no doubt underestimated. Thus, typical reaction mixtures that contained 1% acetonitrile gave erroneously low catalytic rate enhancement by virtue of overestimating the concentrations of free cyclohexaamylose. In the case of 1% acetonitrile solutions only about half of the total cyclohexaamylose is free of occluded acetonitrile and thus available for catalytic cleavage of ester substrates. However, it does not appear possible for us to make quantitative corrections of the reported kinetic data since these corrections rely on certain experimental and calculational details which are not mentioned.

The complexations we have noted here may also be responsible for discrepancies between cyclohexaamylose complexation constants determined by so-called "competitive inhibition" methods and other techniques. For example, we have recently determined the complexation constant of cyclohexaamylose with adamantane carboxylate anion and this value differs substantially from earlier estimates based on inhibition of catalyzed ester hydrolyses re-

(11) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972.

(12) Kitaura, Y.; Bender, M. L. *Bioorg. Chem.* **1975**, *4*, 237–249.

actions.[9] The solvent used in the earlier study contained acetonitrile as a solvent.

Experimental Section

Cyclohexaamylose samples obtained from Aldrich Chemical Co. were allowed to aerate for several days before use. Prepared in this way, the amylose consisted of the hexahydrate form as determined by heating (~110 °C) to constant weight in a vacuum oven. All other materials were reagent grade. pH measurements employed conventional glass and either external calomel or Ag/AgCl reference electrodes and were standardized with 0.05 *m* KHP at the measurement temperature. Electrodes were always equilibrated at the temperature of measurement until no thermal drift (<0.001 pH) was detectable for a period of 20–30 min, a time period comparable to the duration of the subsequent experiment. Readings were obtained after equilibration periods of 2–3 min subsequent to addition of cyclohexaamylose or substrate samples. Mixed solvent solutions were prepared either as bulk stock solutions (2-methyl-2-propanol and cyclohexanol) or volumetrically just before use. Volume additivity was assumed in calculating concentration values.

NMR measurements employed a Bruker HX-270 spectrometer operating at 67.89 MHz for ^{13}C observation. Instrument settings of 30° tip angle, 1.3 s recycle time, and 1K–2K transients were used to scan solutions contained in 10-mm sample tubes which were maintained at 30 ± 1 °C.

Benzoic acid mixed solvent solubility measurements involved dissolution of excess benzoic acid in a suitable quantity of the organic solvent followed by addition of water. The stirred thermostatted mixtures containing large excess of benzoic acid solid were equilibrated for 30 min to 1 h at which time samples were withdrawn, filtered, and titrated with standardized NaOH to the phenolphthalein end point. Subsequent samples taken at intervals of 30 min always yielded the same results within the estimated ±1% precision of our titrations. Most solubility measurements were repeated with fresh solutions and these too gave results to within ±1%. Finally, the water solubility of benzoic acid was determined by withdrawing and titrating samples from a stirred bulk solution over a period of several days.

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Registry No. Cyclohexaamylose, 10016-20-3; acetonitrile, 75-05-8; phenol, 108-95-2; ethanol, 65-17-5; 2-propanol, 67-63-0; 2-methyl-2-propanol, 75-65-0; cyclohexanol, 108-93-0; dioxane, 123-91-1; dimethyl sulfoxide, 67-68-5.

Exciplex Formation in Dodecylammonium Propionate Reversed Micellar Systems

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Abstract: On the basis of the study of some heteroexcimer systems in pure cyclohexane and in dodecylammonium propionate reversed micelles, information on the average localization site of the probes, solubilized in these micelles, is obtained. Probes used were 1-methylnaphthalene, other naphthalene derivatives, and sodium 1-pyrenesulfonate, and quenchers were triethylamine, *m*-dicyanobenzene, and dimethylaniline. For detergent-like probes, which are bound to the micelle, the quenching probability and fluorescence properties are strongly affected by the chain length of the probe, making the determination of the variation of the dielectric constant in the vicinity of the waterpool possible. The influence of water on the quenching process is also investigated, indicating a higher micelle density with increasing water amount.

Introduction

Exciplexes have been studied for many years[1] and are characterized by a sufficiently large dipole moment, that their fluorescence properties are dependent on the polarity of the environment. The formation of radical ions is in strong polar media energetically more favorable than exciplex formation, and exciplex emission is usually not observed. Because of this dependence on the environment, the study of exciplexes in micelles offers an interesting possibility to obtain information on these supramolecular structures.

Although several exciplex studies have been carried out in aqueous micelles,[2] liposomes,[3] and W/O microemulsions,[4] no information on exciplex formation in reversed micelles is available. On the basis of the results obtained in these heterogeneous systems, it appears that this method can give information about the probe localization, an important feature in quenching studies.[5]

In this work, exciplex formation between several naphthalene derivatives and triethylamine (TEA) has been examined in the reversed micellar system dodecylammonium propionate ([DAP] = 0.12 M)/cyclohexane/water ($R = [H_2O]/[DAP] = 1.375$). From earlier measurements in our laboratory,[6] it is known that TEA is a good quencher for 2-methylnaphthalene. However, to make sure that the earlier obtained results can be generalized for

(1) (a) P. Froehlich and E. L. Wehry, "Modern Fluorescence Spectroscopy", Vol. 2, Plenum Press, New York, 1976, p 319; (b) M. Gordon and W. R. Ware, Ed., "The Exciplex", Academic Press, New York, 1975; (c) M. Ottolenghi, *Acc. Chem. Res.*, **6**, 153 (1973); (d) K. B. Eisenthal, *ibid.*, **8**, 118 (1975); (e) H. Leonhardt and A. Weller, *Ber. Bunsenges. Phys. Chem.* **67**, 791 (1963).

(2) (a) M. Grätzel and J. K. Thomas, *J. Am. Chem. Soc.*, **95**, 6885 (1973); (b) H. Masuhara, K. Kaji, and N. Mataga, *Bull. Chem. Soc. Jpn.*, **50**, 2084 (1977); (c) B. Katusin-Razem, M. Wong, and J. K. Thomas, *J. Am. Chem. Soc.*, **100**, 1679 (1978); (d) S. S. Atik and J. K. Thomas, *J. Am. Chem. Soc.*, **103**, 3550 (1981); (e) Y. Waka, K. Hamamoto, and N. Mataga, *Chem. Phys. Lett.*, **53**, 242 (1978); **62**, 365 (1979); *Photochem. Photobiol.*, **32**, 27 (1980).

(3) (a) Y. Waka, N. Mataga, and F. Tanaka, *Photochem. Photobiol.* **32**, 335 (1980); (b) R. McNeil and J. K. Thomas, *J. Colloid Interface Sci.*, **73**, 522 (1980); (c) K. Kano, H. Kawazumi, T. Ogawa, and J. Sunamoto, *Chem. Phys. Lett.*, **74**, 511 (1980); *J. Phys. Chem.*, **85**, 2204 (1981); (d) J. Sunamoto, K. Iwamoto, H. Iwamoto, H. Kondo, K. Kano, and T. Ogawa, *Chem. Lett.*, **1**, 15 (1981); (e) K. Kano, H. Goto, and T. Ogawa, *Chem. Lett.*, **5**, 653 (1981).

(4) S. S. Atik and J. K. Thomas, *J. Am. Chem. Soc.*, **103**, 4367 (1981).

(5) E. Geladé and F. C. De Schryver, *J. Photochem.*, **18**, 223 (1982).

(6) (a) F. Meeus, M. Van der Auweraer, J. C. Dederen, and F. C. De Schryver, *J. R. Neth. Chem. Soc.*, **98**, 220 (1979); (b) F. Meeus, M. Van der Auweraer, and F. C. De Schryver, *J. Am. Chem. Soc.*, **102**, 4017 (1980).

0002-7863/82/1504-6288$01.25/0