apparent pK_a of the group participating in the deacylation. This variation may be attributable to the perturbation of the pK_a induced by the substituted cinnamoyl group (15, 19). A plot of the logarithm of the rate constants in the pH-independent region (k_3^{lim}) against the Hammett σ values gives a straight line (correlation coefficient = 0.994), as shown in Fig. 7. The electron-withdrawing substituent facilitates the reaction and the Hammett ρ value is 1.63. This ρ value suggests that the deacylation proceeds *via* general base catalysis rather than general acid catalysis, because the latter is, in general, independent of polar effects (20). The requirement of the limited conformation around the catalytic site is demonstrated by the finding that the deacylation of denatured cinnamoyl-albumin in 8 *M* urea was retarded.

Although the acylation of albumin with I (k_2) was faster than the spontaneous hydrolysis of I (k_0), as shown in Fig. 4, the deacylation of the cinnamoyl-albumin (k_3 in Fig. 5) is slower than the hydrolysis of I (k_0 in Fig. 4). The deacylation rate is related to the molecular activity (in the past the term "turn over number" has been used) which gives an indication of efficiency of an enzyme (21, 22). In this context, the esterase-like activity rather than the intrinsic esterase may be an appropriate expression for the activity of albumin toward the amide and ester substrates.

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Stability Constants for Complex Formation Between α-Cyclodextrin and Some Amines

ALBERT B. WONG, SHU-FEN LIN, and KENNETH A. CONNORS ˣ

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Abstract □ Complex formation of α-cyclodextrin with 15 amines (including seven 4-substituted anilines) was studied by the potentiometric method, supplemented by direct UV spectrophotometry and a competitive indicator spectrophotometric method. The data were analyzed in terms of 1:1 and 1:2 complexes (amine–cyclodextrin ratios) and the stability constants K_{11a}, K_{12a}, K_{11b}, and K_{12b} were evaluated; the subscripts indicate the stoichiometry and conjugate acid–base form. For all amines K_{11b} was greater than K_{11a} and K_{12a} was 0. On the basis of the relationship of complex stability to amine structure, it was concluded that the primary binding site in anilines is the 4-substituent.

Keyphrases □ Complex formation—of α-cyclodextrin with amines, determination of the stability constants and binding sites □ α-Cyclodextrin—complex formation with amines, determination of stability constants and binding sites □ Amines—complex formation with α-cyclodextrin, determination of stability constants and binding sites

Cycloamyloses (also called cyclodextrins) are cyclic oligomers containing six or more D-glucose units linked 1 → 4; they are produced by the action of *Bacillus macerans* amylase on starch. The six- and seven-unit substances are called cyclohexaamylose (α-cyclodextrin) and cycloheptaamylose (β-cyclodextrin), respectively. These molecules are doughnut shaped, and their possession of a cavity of fixed size and shape has led to considerable interest in their chemical properties. The production, purification, and chemistry of the cycloamyloses have been reviewed (1–4).

Any molecule smaller than the cavity of a cyclodextrin can enter the cavity and there undergo noncovalent interaction with the atoms lining and rimming the cavity. The resulting association product is called an inclusion complex. The cyclodextrin is thus a host for the smaller (guest) molecule. The dimensions of the α-cyclodextrin cavity permit the inclusion of many mono- and disubstituted benzene derivatives. A 1:1 stoichiometry is commonly observed (and often assumed in experimental studies), but it has now been well established that 1:2 complexes (*i.e.*, 1 substrate:2 cyclodextrins) may exist in some systems[1] (5–9).

The present paper is one of a series that describes

[1] The substrate (S) is the guest; the cyclodextrin (ligand, L) is the host. Stoichiometric ratios are given in the form SL (1:1) and SL_2 (1:2).

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Table I—Stability Constants for α-Cyclodextrin Complexes of 4-Substituted Anilines at 25° [a]

X[b]	K_{11b}/M^{-1}	K_{12b}/M^{-1}	K_{11a}/M^{-1}
NH_2	2.3 (0.10)	2.1 (1.0)	0
OCH_3	6.7 (0.14)	−0.5 (0.3)	0
CH_3	57.6 (0.34)	3.91 (0.09)	37.1 (1.1)
H	8.8 (0.12)	−0.39 (0.39)	0
COO^- [c]	9.0	0	—[d]
Cl	251 (10.0)	0.12 (0.16)	68.6 (3.3)
COOH[c]	1341	0	—[d]
CN	451 (33.7)	—[d]	—[d]
NO_2	635 (34.4)	—[d]	—[d]

[a] Standard deviations in parentheses. [b] X in X—C_6H_4—NH_2. [c] From Ref. 9. [d] — not determined.

measurement of stability constants for complex formation between α-cyclodextrin and many substrates in aqueous solution, with the possible presence of 1:2 complexes being taken into account. The interpretation of the results is aided and unified by a model of the complexing that describes the substrate as a species having two possible binding sites (10). Most of the substrates are 1,4-disubstituted benzenes and many of them are ionizable, so the conjugate acid and base forms constitute separate guest species. In this paper a series of amines was studied as substrates. The experimental stability constants are symbolized by K_{11a}, K_{12a}, K_{11b}, and K_{12b}, the subscripts denoting complex stoichiometry and the conjugate acid–base form of the substrate.

Since these substrates are weak bases, the potentiometric method, which has been described in detail earlier (9), was applied. This technique was supplemented by UV spectrophotometry and a competitive spectrophotometric indicator method (11).

EXPERIMENTAL

Materials—α-Cyclodextrin[2] was dried at 95° for 48 hr. The amines were from commercial sources[3]; they were purified by recrystallization or distillation, the melting and boiling points being in good agreement with literature values (12, 13).

Procedures—The potentiometric method was used as described in detail earlier (9), except that the amine substrates were half-neutralized with 0.1 *N* HCl. The method of treating the data to obtain the stability constants was explained in detail. K_{11a} for 4-toluidine was measured by the competitive spectrophotometric method, using methyl orange as the indicator. The pK_a values of 4-nitroaniline and 4-cyanoaniline are 1.00 and 1.74, respectively (14, 15)—too low for these systems to be studied potentiometrically. Their K_{11b} values were measured by UV spectrophotometry. For both systems, 1:1 stoichiometry was suggested by the observation of isosbestic points. 4-Nitroaniline yielded the same K_{11b} value from measurements at 350 and 410 nm. The 4-cyanoaniline system was studied at 260 nm. All results reported here are at 25.0° and 0.1 *M* ionic strength.

RESULTS

Seven 4-substituted anilines were studied, most of them by the potentiometric method. The stability constants found for these amines are given in Table I. For all systems K_{12a} was 0. The conjugate acid of *p*-phenylenediamine (4-aminoaniline) has $pK_{a1} = 2.89$ and $pK_{a2} = 6.16$ (16), so that measurement of pK_{a2} left the other amino group (the X in Table I) in its base form. K_{12b} is not significantly different from zero at the 95% level for X = NH_2, OCH_3, H, Cl (though for X = NH_2, K_{12b} is significant at somewhat lower confidence levels).

Eight other aromatic or heterocyclic amines were studied potentiometrically. The results are listed in Table II. For all of these systems, K_{12a} was 0. Benzylamine, 4-*tert*-butylpyridine, and isoquinoline yielded significant K_{12b} values. The K_{12b} value for *N*-methylimidazole, though small, appears to be significant; however, when this system was treated as a Case II system (9), for which $K_{12b} = 0$ by definition, it gave a correlation coefficient (R^2) of 1.00, compared with 0.03 for the Case III (finite K_{12b}) system. Therefore, it was concluded that $K_{12b} = 0$ for this system, and, evidently, for the remaining amines in Table II[4].

Table II—Stability Constants for α-Cyclodextrin Complexes of Amines at 25° [a]

Amine	K_{11b}/M^{-1}	K_{12b}/M^{-1}	K_{11a}/M^{-1}
Benzylamine	17.4 (0.35)	4.0 (0.26)	0
Phenethylamine	26.4 (0.17)	−0.44 (0.16)	0
Imidazole	16.3 (0.23)	0.05 (0.37)	0
N-Methylimidazole	13.4 (0.09)	0.81 (0.23)	0
4-Nitroimidazole	49.8 (0.81)	−0.0006 (0.16)	4.0 (0.25)
4-*tert*-Butylpyridine	84.7 (6.0)	20.1 (1.8)	0
Quinoline	28.6 (0.73)	0.63 (0.48)	0
Isoquinoline	22.7 (0.95)	10.8 (0.76)	0

[a] Standard deviations in parentheses.

DISCUSSION

Binding Site Model—Most of the substrates studied in the present investigation, as well as those in earlier studies, can be viewed as substrates possessing two potential binding sites. In 1,4-disubstituted benzenes, for example, the 1 and 4 positions are the possible sites for binding to the cyclodextrin cavity. For such a substrate, having binding sites X and Y, it was shown (9) that:

$$K_{11} = K_X + K_Y \qquad \text{(Eq. 1)}$$

and

$$K_{12} = \frac{aK_XK_Y}{K_{11}} \qquad \text{(Eq. 2)}$$

In these equations K_{11} and K_{12} are the experimental stability constants for 1:1 and 1:2 complex formation, K_X and K_Y are binding constants for 1:1 complexation of the ligand at sites X and Y, and a is a parameter that describes interaction between the two sites in a 1:2 complex. According to Eq. 1, the experimental K_{11} is the sum of binding site constants for isomeric 1:1 complexes. The 1:2 complex is formed by adding a second cyclodextrin to either of the preformed 1:1 complexes; according to Eq. 2, K_{12} can be zero only if $a = 0$ or if one of the site binding constants is zero.

If the two binding sites are identical, as in a symmetrical 1,4-disubstituted benzene, then $K_X = K_Y$, and Eqs. 1 and 2 give:

$$K_X = K_{11}/2 \qquad \text{(Eq. 3)}$$

and

$$a = 4K_{12}/K_{11} \qquad \text{(Eq. 4)}$$

For such a substrate, the binding site constant K_X and the interaction parameter a are obtainable.

The interpretation of experimental stability constants in terms of this model focuses attention on the binding sites and makes use of some chemical assumptions to assign binding constants to binding sites. It is postulated that (aside from binding site size and shape factors) the primary feature of a binding site contributing to complex stability is its electron density. Also favoring complex stability is the polarizability of the binding site, whereas complexing is weakened by high site polarity. The net effect of these factors determines the binding site constant for binding in a given solvent at constant temperature.

Anilines—The K_{11b} values in Table I show a rough trend with electron-attracting ability by the X-substituent, indicating that the X-site is the principal binding site. 4-Methylaniline appears to bind anomalously strongly. The binding of 4-aminobenzoic acid may be enhanced by hydrogen bonding. The K_{11b} values are correlated by:

$$\log K_{11b} = 1.70\sigma + 0.05R_D \qquad \text{(Eq. 5)}$$

[2] Sigma Chemical Co., Lot Nos. 20F-0507 and 29C-0425.

[3] Mallinckrodt Chemical Co.; Aldrich Chemical Co.; Eastman Organic Chemicals.

[4] Many of the constants in Tables I and II are quite small. For such systems the diagnostic plots described in Ref. 9 may be ambiguous because of the considerable relative experimental error and the limited range of the binding isotherm that is accessible to observation.

Table III—Comparison of K_{11b} for Anilines and K_{11a} for Phenols[a]

X[b]	K_{11b} (aniline)[c]	K_{11a} (phenol)[d]
OCH_3	6.7	0
CH_3	57.6	0
H	8.8	0
COO^-	9.0	16.6
Cl	251	272
COOH	1341	1130
CN	451	158
NO_2	635	245

[a] At 25°. [b] X in X—C_6H_4—NH_2 or X—C_6H_4—OH. [c] From Table I. [d] From Ref. 11.

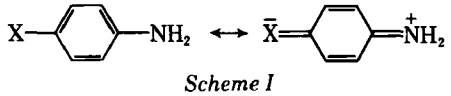
where σ is the Hammett substituent constant of X and R_D is the molar refraction of X—C_6H_5. The standard deviation is 0.39 in log K_{11b}.

K_{11b} is larger than K_{11a} for all amines, a result that is consistent with the postulates that complex stability is enhanced by high site electron density and polarizability and decreased by high polarity.

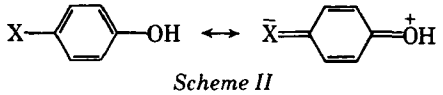
Since 4-aminoaniline is symmetrical, Eqs. 3 and 4 are applicable, giving $K_X = 1.15$ as the binding constant for the 4-amino group in this substrate. Calculation of the a value with Eq. 4 does not seem justified, because of the large uncertainty in K_{12b}.

Since the X group in 4-aminoaniline is NH_2, the most electron-releasing group in Table I, any other X group will be even less effective in increasing electron density at the aniline NH_2 site. Thus the binding site constant for binding to the amino site should be a maximum for 4-aminoaniline (for which it is 1.15 M^{-1}). Since K_{11b} is, by Eq. 1, the sum of the binding constants at the two sites and the binding constant at the amino site has been shown to be 1.15 or smaller, the K_{11b} values in Table I can (except for X = NH_2) be essentially completely assigned to binding at the X-substituent site.

If this assignment is correct, an interesting comparison can be made between K_{11b} for anilines and K_{11a} for the corresponding phenols, for it was concluded earlier (11) that K_{11a} for phenols can be ascribed to binding at the 4-substituent site[5]. The electronic distribution of neutral anilines and phenols should be similar, as shown in Schemes I and II.



Scheme I



Scheme II

Therefore, it is expected that K_{11b} for anilines should be approximately equal to K_{11a} for the corresponding phenols. These quantities are compared in Table III, which shows that the agreement is reasonable in terms of this simple argument.

[5] Except for ^-OOC—C_6H_4—OH, which may bind at the hydroxy site (2).

The stability constant K_{11a} presumably describes binding at the X-site, since the positively charged protonated amine site will on all counts (reduced electron density, reduced polarizability, and increased polarity) not be expected to undergo any complexation. That K_{11a} is zero for several systems indicates that electron density at the X-site is reduced sufficiently, in the cation, that no binding occurs even at this site.

Other Amines—The data in Table II show the general pattern $K_{11b} > K_{11a}$ for amines. Many structural types are represented, so interpretations of most of the results will require more extended and systematic variation of structures to establish correlations of structure with complex stability. Some obscure points, for example, are the difference in 1:2 complex behavior for quinoline and isoquinoline and the appearance of a finite K_{12b} for benzylamine but not for aniline or phenethylamine[4]. Some results seem reasonable, however; for example, the larger K_{11b} for 4-nitroimidazole compared with imidazole suggests that the nitro group is the principal binding site. Moreover, this is the only compound in Table II that possesses a finite K_{11a}, probably because the powerfully electron-withdrawing nitro group opposes the electron withdrawal by the protonated amine, leaving sufficient electron density at the binding site to result in significant complexation.

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