Effects of Various Salts on the Binding of Indole Compounds with Bovine Albumin*

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RAPIER H. McMENAMY, MICHAEL I. MADEJA, AND FRANK WATSON

From the Departments of Biochemistry and Surgery, State University of New York at Buffalo, and the Edward J. Meyer Memorial Hospital, Buffalo, New York 14214

SUMMARY

Influences of different concentrations of salts (0.002 to 1.25 eq per liter) on the binding of skatole and acetyl-L-tryptophan at the primary binding site of bovine plasma albumin have been studied by equilibrium dialysis. With the exception of Na₂-ethylenediaminetetraacetate and ethylenediammonium aspartate, which have little effect, all salts in the concentration range of 0.005 to 0.1 eq per liter inhibit the binding of acetyl-L-tryptophan; their order of effectiveness, which is invariant with salt concentration, is KClO₄ > KSCN > KI > LiCl > CaCl₂ > KF > KCl > NaCl > ethylenediammonium chloride > K₂SO₄. With skatole the addition of halide-like salts (except KF) in the concentration range of 0 to 0.05 eq per liter increases the binding markedly. The order at 0.002 eq per liter solute is KClO₄ > KSCN > KI > ethylenediammonium chloride > KCl > LiCl > CaCl₂ (this order is consistent with increased skatole binding with increased salt anion binding). Between 0.05 and 0.25 eq per liter binding of skatole decreases with further addition of KCl, and KSCN, remains approximately constant with further addition of KI, but increases with further addition of the Cl⁻ salts. Other solutes have no effect (Na₂-ethylenediaminetetraacetate, ethylenediammonium aspartate, K₂SO₄ and glycine), or a small enhancing effect (KF) on skatole binding. Between 0.25 and 1.25 eq per liter the addition of most salts increases the binding of skatole. The salt concentration binding profile of β-3-indolethanol in KSCN solutions is similar to that of acetyl-L-tryptophan, implying that the charge of the acetyl-L-tryptophan is not the element which leads to the differences between its binding and that of skatole. Effects of SCN⁻ and Cl⁻ on acetyl-L-tryptophan-albumin binding show direct competitive inhibition as well as anomalous ionic strength effects.

It is concluded that the primary influence of salts is against the polar elements of the binding center.

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That salts have a specific effect on the solubility of proteins is a well known observation. A specific influence of solvents on protein denaturation (1) and alteration of enzyme activity (2) have also been shown. The solubility of a modified peptide, acetyltetradecylglycine ethyl ester, has been extensively studied in solutions of different salts (3, 4) and it has been found that the increase in solubility of the modified peptide generally follows the order in which the salts increase denaturation or unfolding of the tertiary structure of the proteins. The effect of salts on the melting temperature transitions of ribonuclease, collagen, and DNA (5-7) has been studied. The order of salts in decreasing the temperature of transition closely follows the order of effectiveness described for protein structural changes.

Although a denaturation order for salts has been reasonably well described, the basis or mechanism responsible for specific salt effects on proteins is still largely obscure. For the most part, studies of salt effects on proteins have been evaluated by observation of gross changes in the properties of the proteins. This has been a drawback in that one is generally not able to determine the nature of the positions at which the salts exert their influences on protein structure—whether hydrophobic or hydrophilic locations are involved, whether hydrogen bond breaking and coulombic force disturbances play roles, whether solvent alterations are important, etc.

The binding of indole compounds with albumin offers an opportunity to evaluate salt interactions at a specific location on protein. The fact that the indole ligands bind almost entirely at one site on the albumin permits small changes in binding to be readily detected and to be assigned unequivocally to the one site location. Considerable information is already available on the nature of the protein-indole binding site. Furthermore, ligands with diverse properties can be used in binding studies to evaluate the effects of salts. The binding of skatole, an apolar ligand, of β-3-indolethanol, an apolar ligand with a small polar group, and of acetyl-L-tryptophan, a ligand with polar and apolar substituents, including a negative charge, has therefore been determined in the presence of different salts at different concentrations. To avoid confusion, the term “ligand” refers to the indole compounds, and the term “solute” is restricted to salts and other compounds added to the protein solutions.
EXPERIMENTAL PROCEDURE

Materials

Bovine plasma albumin (Armour, Lot Y20007) was the same preparation as used in other studies (8). It was dialyzed extensively against 1.0 mM EDTA and deionized by passage through a mixed bed resin column. By electrophoresis it contained less than 2% nonalbumin, and by ultracentrifugation it contained less than 2% dimer. Salts and other solutes were at least reagent grade and were used as obtained from the supplier. Skatole and acetyl-L-tryptophan were obtained from Mann Research Laboratories and were recrystallized from water and ethanol, respectively. Tryptophol (β-3-indolyethanol) was obtained from Calbiochem and was recrystallized from water.

Methods

Solubility Studies—Solubility studies were conducted essentially as previously described (8). At least duplicate saturation experiments were conducted at each salt concentration. The amount of the indole crystals added was varied over a range of 3-fold. Duplicate experiments agreed within 6%.

Binding Studies—These were conducted as previously described by equilibrium dialysis at 4° and 18° with the use of a thin layer technique to shorten the time necessary for equilibrium (9, 10). With this technique 2 ml of protein and ligand solution were placed inside the dialysis bag and 3 ml of salt solution outside. Albumin concentrations were 0.5 ± 0.05 mM and were determined by refractometry (8). At each salt concentration the association constant was determined from five dialysis experiments, wherein the amount of ligand added was approximately 0.35, 0.50, 0.67, 1.0, and 1.5 times the moles of albumin added. Ligand concentrations were determined by absorbance or spectrofluorometry measurements (10). Donnan corrections were computed as previously described (8, 11). The moles of ligand bound per mole of protein, \( n_2 \), were determined from the difference between the moles of ligand added and moles of ligand in free solution, divided by moles of protein present. All studies were conducted at pH 6.1 to 6.5. Hydroxides of the salt cation were added to adjust the pH.

The method of obtaining \( k'_1 \), the apparent binding constant, and \( n_1 \), the number of binding sites with the binding constant \( k'_1 \), is described in detail elsewhere (10, 11). In this method the binding data were plotted as \( F/A \) against \( v \), wherein \( A \) is the concentration of free ligand. Values for the primary site, designated \( k'_1 \) and \( n_1 \), were then obtained from the plot with the best fit technique by Equation 1. In no case was it necessary to assume more than two sets of sites in order to fit the plot. The secondary sites were only crudely approximated by arbitrarily assigning \( n_2 = 10 \). They are usually not recorded.

\[
F = \frac{n_1 k'_1 A}{1 + k'_1 A} + \frac{n_2 k''_1 A}{1 + k''_1 A} \tag{1}
\]

The accuracy of the curve-fitting technique is illustrated by some examples in Fig. 1. For \( k'_1 \) values less than \( 5 \times 10^{-6} \) the standard error of \( k'_1 \) for acetyl-L-tryptophan was estimated as ± 6%. Because of the very low concentration of the ligand involved \( k'_1 \) was less accurate at values higher than \( 5 \times 10^{-6} \). Except where binding at the primary site was low the \( k'_1 \) values for skatole were obtained at approximately the same accuracy as those of acetyl-L-tryptophan. With skatole, secondary site binding did not decrease as fast as that at the primary site, and in the case of low binding it became difficult to separate the contributions of \( n_1 \) and \( n_2 \) site sets. An example of a low binding condition is that of the binding of skatole in 0.05 M K2SO4 (Fig. 1). Values for \( k'_1 \) would never be greater than the \( F/A \) axis intercept; however, if the arbitrary selection of \( n_2 = 10 \) was removed, they could well be much less than this intercept. With tryptophol the primary binding site was always distinguishable, although secondary site binding was usually appreciable. Since the primary binding constant is the only binding constant considered in this paper, the subscript "1" is omitted in all subsequent references to this binding constant. The units of \( k'_1 \) are always moles per liter (M⁻¹).

RESULTS

Solubility of Skatole in Various Salt Solutions—The results of the solubility studies with skatole are reported in Table 1. Except for Na₂EDTA and KSCN solutions, in which the solubility of skatole is almost the same as in water, the solubility of skatole decreases with the addition of salts. At equivalent solute concentrations, the solubility of skatole is approximately the same in solutions of KCl, LiCl, CaCl₂, and KF; it is lowest in K₂SO₄. The solubility of tryptophol was determined at 4° in several salt solutions (all at 0.2 eq per liter). In EDTA, KSCN, CaCl₂, KCl, and H₂O it was 24.8, 26.2, 23.2, 22.1, and 26.3 mM, respectively. The effects of salts on the solubility of this ligand are in the same order as on skatole.

Salt Effects on Binding of Skatole and Acetyl-L-tryptophan with Albumin—Figs. 2 to 5 show the effect of various salts on the binding of skatole and acetyl-L-tryptophan with bovine albumin. The concentrations of salts are expressed as equivalents per liter. By comparison of the salts with common cations it is apparent that some anions which are halide or halide-like (Cl⁻, Br⁻, I⁻, SCN⁻, and CLO₄⁻) enhance the binding of skatole (especially at low salt concentrations) whereas they decrease the binding of acetyl-L-tryptophan. These enhancing and inhibitory effects correlate directly with the order of affinity of the salt ions for albumin (12-15). There is also a series of anions which have little effect on the binding of skatole; these are F⁻, aspartate⁻, SO₄²⁻, and EDTA. With the binding of acetyl-L-tryptophan these latter ions have variable effects; with EDTA and aspartate binding of acetyl-L-tryptophan is little affected, whereas with SO₄²⁻ (on a molar basis) and F⁻ an inhibitory effect is found which is almost as large as found with Cl⁻. The order of increasing inhibition of acetyl-L-tryptophan binding by anions also agrees reasonably well with the order of the disruptive effects of the anion on protein structure (1-7). The latter is obviously not true for the effect of the salt ions on skatole binding. The effect of cations on the binding of both skatole and acetyl-L-tryptophan is consistent with their order of denaturation ability on proteins (3); i.e. by comparison of their Cl⁻ salts, both Ca²⁺ and Li⁺ are more potent inhibitors (and stronger denaturants) than K⁺, which is a slightly more potent inhibitor than Na⁺ (11). As for a neutral substance, glycine, a dipolar compound, has no effect on the binding of skatole. Similarly, glycine has been shown to have no effect on the binding of acetyl-L-tryptophan (8). There is almost no correlation between the solubility of skatole in the salt solutions and its binding with albumin in the same solutions.

Figs. 2 to 5 also show that, whereas the order of anion effects on the binding of acetyl-L-tryptophan does not change with change in salt concentration, the order of the anion effects on the
binding of skatole does indeed depend on the salt concentration. Thus, at low salt concentrations (0.05 M and less), SCN\(^{-}\) and ClO\(_4\)\(^{-}\) markedly enhance skatole binding, whereas at higher concentrations (>0.05 M) their enhancing effect diminishes so that skatole binding is actually reduced with further addition of these ions. I\(^{-}\) also induces an early enhancing effect which, however, levels off at intermediate I\(^{-}\) concentrations. Cl\(^{-}\), on the other hand, shows a continuous enhancing effect with all increases in its concentration. One finds, therefore, that at concentrations of 0.002 eq per liter skatole binding is favored by

![Graph of skatole binding with bovine albumin at different salt concentrations, pH 6.1 to 6.5. EDNH\(_2\) ASP, ethylenediammonium aspartate.](image)

**Table I**

| Solubility of skatole in various salt solutions
<table>
<thead>
<tr>
<th>Solute concentration</th>
<th>KCl</th>
<th>LiCl</th>
<th>KSCN</th>
<th>KF</th>
<th>K(_2)SO(_4)</th>
<th>Na(_2)EDTA</th>
<th>CaCl(_2)</th>
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</thead>
<tbody>
<tr>
<td>eq/liter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.2 (3.5(^{\circ}))</td>
<td>1.065</td>
<td>1.104</td>
<td>0.980</td>
<td>1.065</td>
<td>1.202</td>
<td>1.047</td>
<td>1.066</td>
</tr>
<tr>
<td>0.2 (18(^{\circ}))</td>
<td>1.139</td>
<td>1.191</td>
<td>1.063</td>
<td>1.195</td>
<td>1.186</td>
<td>1.049</td>
<td>1.101</td>
</tr>
<tr>
<td>1.25 (3.5(^{\circ}))</td>
<td>1.709</td>
<td>1.681</td>
<td>1.087</td>
<td>1.848</td>
<td>2.725</td>
<td>1.064</td>
<td>1.724</td>
</tr>
<tr>
<td>1.25 (18(^{\circ}))</td>
<td>1.800</td>
<td>1.600</td>
<td>1.053</td>
<td>1.647</td>
<td>2.510</td>
<td>1.025</td>
<td>1.642</td>
</tr>
</tbody>
</table>

* This ratio is the activity coefficient of skatole in the test solution. The solubilities of skatole in water at 3.5\(^{\circ}\) and 18\(^{\circ}\) are 1.704 and 2.749 mm, respectively.

* Eight-tenths equivalent per liter.

* One-half equivalent per liter.

![Graph of skatole binding with bovine plasma albumin at different salt concentrations, pH 6.3 to 6.5, 4\(^{\circ}\). In all instances k\(_{1}'\) is X 10\(^{-3}\), n\(_1\) = 1, k\(_{2}'\) is X 10\(^{-4}\), n\(_2\) = 10. Skatole binding with bovine plasma albumin, ----, KCl, 0.1 M, k\(_{1}'\) = 6.3, k\(_{2}'\) = 6. ▲, CaCl\(_2\), 0.05 M, k\(_{1}'\) = 2.5, k\(_{2}'\) = 6. ●, KF, 0.005 M, k\(_{1}'\) = 1.1, k\(_{2}'\) = 6. □, K\(_2\)SO\(_4\), 0.05 M, k\(_{1}'\) = 0.55, k\(_{2}'\) = 7. Acetyl-L-tryptophan binding with bovine plasma albumin, -----, KI, 0.005 M, k\(_{1}'\) = 4.2, k\(_{2}'\) = 1. ○, KSCN, 0.05 M, k\(_{1}'\) = 0.45, k\(_{2}'\) = 0.2.](image)

**Fig. 2.** The binding of skatole with bovine albumin at different salt concentrations, pH 6.1 to 6.5. EDNH\(_2\) ASP, ethylenediammonium aspartate.
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FIG. 4. The binding of acetyl-L-tryptophan with bovine albumin at different salt concentrations, 4°C, pH 6.1 to 6.5. Not shown are several $k'$ values for ethylenediamine chloride solutions, in which inhibition is slightly less than in KCl solutions, KF solutions in which inhibition is slightly greater than KCl solutions, and ethylenediamine sulfate solutions in which inhibition is the same or slightly less than in Na$_2$EDTA solutions. At very low salt concentrations the Donnan corrections for KCl, KSCN, and KI were quite large and not very certain because of the large amount of anion building. Values of $k'_1$ in these regions may be in some error because of this.

FIG. 5. Same as Fig. 4 except at 18°C

FIG. 6. The inhibition of acetyl-L-tryptophan-albumin binding by Cl$^-$ and SCN$^-$ in 0.2 M EDTA, pH 6.3 to 6.4. ○, Cl$^-$, 4°C; △, Cl$^-$, 18°C; □, SCN$^-$, 4°C; ×, SCN$^-$, 18°C, $k_0$ = 70 (4°C and 18°C); $k_{des}$ = 440 (4°C) and 480 (18°C). In the presence of Cl$^-$, $\lambda_Y$ = $1.52 \times 10^8$ (4°C) and $6.6 \times 10^8$ (18°C). For Cl$^-$ concentrations of 0.05, 0.02, 0.01, 0.0033, and 0.001 M, the following values were taken for $-Z_p$: 17.14, 12, 11, and 10.5, respectively. For SCN$^-$ concentrations of 0.02, 0.01, 0.0033, and 0.001 M, the $-Z_p$ values were taken as 19, 15, 13, and 11, respectively; $w$ was taken as 0.0221.

Anions in the order ClO$_4^-$ $>$ SCN$^-$ $>$ I$^-$ $>$ Cl$^-$; at concentrations 0.25 eq per liter, on the other hand, binding is favored by anions in the order of Cl$^-$ $>$ I$^-$ $>$ ClO$_4^-$ $>$ SCN$^-$; in almost every instance, $k'_1$ is increased when solute concentrations are increased from 0.25 to 1.25 per liter. A good part of this increase seems attributable to changes in the activity coefficient of skatole in this higher salt region (Table I).

The secondary site binding is usually not evaluated. However, it is apparent qualitatively that salts do not affect the secondary site binding of skatole to the extent that they affect the primary site. This can be gleaned from Fig. 1. Secondary site binding is essentially the same for all four of the skatole studies shown there. With acetyl-L-tryptophan secondary site binding is affected in a manner very similar to the primary site binding. This implies that salt ions inhibit, to the same relative degree, binding at both types of sites for this ligand. This is illustrated by the two binding studies of acetyl-L-tryptophan reported in Fig. 1.

EDTA Binding with Albumin—The absence of inhibition by EDTA suggests that EDTA probably has a very low affinity for albumin. To evaluate this further, isoelectric point determinations were undertaken with horizontal paper electrophoresis. To prevent evaporation and to control temperature, the paper was enclosed between two stainless steel plates covered with Saran Wrap (The Dow Chemical Company, Midland, Michigan). Tris-DNB (Tris reacted with dinitrofluorobenzene) was used as a marker to measure endomosis. In 0.2 M EDTA the isoelectric point was found to be $\sim$4.75. As a check the isoelectric point in 0.1 M acetate was found to be $\sim$4.70 which agrees with the value found by moving boundary electrophoresis. Assuming that Na$^+$ does not bind to albumin it is estimated in 0.2 M EDTA, pH 4.75, that approximately 2.5 EDTA molecules are associated with the albumin. That this is a very small amount becomes evident from the computation that in 0.2 M Cl$^-$ approximately 25 Cl$^-$ ions are estimated to be associated with albumin at its isoelectric...
point. The EDTA ion does not appear to be inhibitory to the binding of acetyl-L-tryptophan.

**Competitive Inhibition of Cl⁻ and SCN⁻ against Acetyl-L-tryptophan Binding**—In view of the small influence of EDTA on the binding of acetyl-L-tryptophan and skatole, binding studies were undertaken in EDTA solutions with variable Cl⁻ and SCN⁻ concentrations in order to determine the inhibitory constants of the latter ions. In Fig. 6 (left) the inhibitory effect of Cl⁻ on acetyl-L-tryptophan binding is evaluated by plotting (Cl⁻)⁻δw2ζ₂wζCl⁻ against (k' Cl⁻ζ₂wζCl⁻)⁻¹. In accordance with competitive inhibitory relationships (11, 12), expressed in Equation 2, the intercepts on the abscissa and ordinate axes in this plot are −1/k_i⁻ and 1/k_i²⁻⁻₀, respectively.

\[
(\text{Cl}⁻)^{-δw2ζ₂wζCl⁻} = \frac{k'_{\text{AT}}}{k_{\text{AT}} - k'_{\text{Cl}⁻}} \times \frac{1}{k^0_{\text{Cl}⁻}} \tag{2}
\]

In this equation, the electrostatic interaction parameter, \(w\), is derived from the relationship \(w = 0.115 + 10.53\mu/1 + 10.53\mu\) where \(\mu\) is the ionic strength (13); \(z_{\text{Cl}⁻}\), \(z_{\text{AT}}\), and \(z_{\text{Cl}⁻\text{AT}}\) are the charges on the albumin, Cl⁻, and acetyl-L-tryptophan, respectively; \(k'_{\text{AT}}\) is the apparent binding constant for acetyl-L-tryptophan, and \(k'_{\text{Cl}⁻\text{AT}}\) and \(k'_{\text{Cl}⁻}\) are the intrinsic binding constants for the two ligands at the condition of 0.2 m EDTA. The right side of Fig. 6 is a similar type study wherein the SCN⁻ inhibition of the acetyl-L-tryptophan-albumin binding is evaluated in the same manner as that of Cl⁻. These studies show that the inhibitory constants of the latter ions against acetyl-L-tryptophan-albumin binding in the presence of 0.2 m EDTA are indeed independent of the Cl⁻ and SCN⁻ concentrations. This differs from what is found in the absence of EDTA in which the inhibitory constant found for Cl⁻ at low Cl⁻ concentration is much higher than that at high Cl⁻ concentrations (9). Since the ionic strength varies markedly in the EDTA-free studies, and varies little in the presence of EDTA, the changes in the inhibitor constants in EDTA-free solutions can be attributed to the ionic strength variations. Studies of SCN⁻ binding with albumin in EDTA solutions also support a large ionic strength involvement in the binding of anions.

**Effect of SCN⁻ on Tryptophol and Skatole Binding**—The effects of SCN⁻ on the skatole-albumin binding and tryptophol-albumin binding, both in the presence and absence of EDTA, are shown in Fig. 7. EDTA has little effect in these studies, further supporting its role as an "inert salt." Interestingly, the effect of SCN⁻ on the tryptophol-albumin binding is very similar to the inhibitory effect of SCN⁻ on the acetyl-L-tryptophan-albumin binding.

**Entropy Changes of Association**—ΔS° values were computed for skatole and acetyl-L-tryptophan from some of the binding constants at 4° and 18°. With LiCl, CaCl₂, KI, and KSCN solutions ΔS° for skatole was found to increase with increases in salt concentration. Values ranged from 0 to −20 cal per mole degree at 0.002 eq per liter, to 15 to 5 cal per mole degree at 1.25 eq per liter. With KCl, LiCl, and CaCl₂ solutions ΔS° for acetyl-L-tryptophan binding became more negative with increases in salt concentration. Values ranged from 23 to 15 cal per mole degree at 0.005 eq per liter, to −7 to −3 cal per mole degree at 0.1 eq per liter. ΔS° values for other salts were rather dispersed.

1 R. H. McMenamy, M. I. Madeja, and F. Watson, unpublished data.
structure of the protein and salt ions could presumably interfere with these forces. The concept of carboxyl group interactions with other groups on the protein was suggested by Saroff (19) several years ago on the basis of Cl\(^{-}\) binding and potentiometric titration data. Although the possible presence of this type of interaction force must be kept in mind there is little evidence which actually supports its playing a major role in protein stability. Plasma albumin for example, is still structurally compact even under conditions in which 50 anions or more are bound.\(^2\) If this binding caused a loss of \(-50\) to \(-100\) kcal per mole energy between adjacent peptide chains some changes in protein shape undoubtedly would have occurred.

The fact that the salt concentration binding profile of tryptophol (a neutral ligand) shows inhibition by SCN\(^{-}\) in a manner similar to that of acetyl-L-tryptophan (a negatively charged ligand) implies that the negative charge of the ligand is not essential to the inhibitory role of the salt. These studies indicate that the \(-\text{OH}\) group on tryptophol is probably the moiety inhibited by SCN\(^{-}\) in the same manner as the \(-\text{C(CH}_3\text{CONH})\)-H—COO\(^{-}\) group on acetyl-L-tryptophan is the moiety inhibited by SCN\(^{-}\). It is furthermore of some interest that the inhibition of tryptophol by SCN\(^{-}\) is essentially the same in the presence and absence of EDTA. Analogous studies of acetyl-L-tryptophan binding in the presence of Cl\(^{-}\) show that EDTA markedly affects the inhibition by Cl\(^{-}\) at low Cl\(^{-}\) concentrations. These latter effects have been attributed to anomalous ionic strength influences on the negatively charged ligand acetyl-L-tryptophan. The fact that the binding of tryptophol, a neutral compound, is not influenced by EDTA in this manner supports the previous assignment of the anomalous effect to ionic strength influences. The fact that EDTA has little or no effect on the binding of skatole, acetyl-L-tryptophan, or tryptophol and the fact that glycine and ethylenediammonium acetate have little or no effect on the binding of skatole and acetyl-L-tryptophan have interesting implications. The positively charged nitrogen group on these solutes is undoubtedly responsible for their skatole, acetyl-L-tryptophan, or tryptophol and the fact that glycine and ethylenediammonium acetate have little or no effect on the binding of skatole and acetyl-L-tryptophan have interesting implications. The positively charged nitrogen group on these solutes is undoubtedly responsible for their small interaction with albumin. Bickle and Bovet (20) have studied the interaction of a large number of amines with albumin and find binding to occur only when there are substantial apolar radicals attached to the ligand. In earlier studies, we also found that tryptamine and L-tryptophan did not bind with albumin except at high pH values at which positive charge of the amino group was removed. In some instances the binding of a charged amino group is unfavorable to the extent of 5 kcal per mole energy (11). The repulsion of the charged amino group does not seem to depend on whether the amino group exists as part of a dipolar ion. To our knowledge no monovalent cation binds with albumin. Whether the poor binding ability of charged nitrogen groups is due to a general inability of monovalent cations to bind with albumin or whether it is due to the nitrogen group itself seems to be an open question. It is to be noted that the net charge on both aspartate and EDTA is negative. A positive charge at any position on the ligand therefore seems to lead to an unfavorable binding condition.

Finally, the fact that the effect of salts on the two ligands is almost exactly opposite in each instance (i.e. salts which enhance skatole binding inhibit acetyl-L-tryptophan binding and vice versa) makes any postulate for solvent structure alteration as an explanation of the salt influences seem of dubious value. Changes in water structure due to the presence of salts would seem, at most, to be of only secondary importance in the present studies.

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