Thermodynamic Values Related to the Association of L-Tryptophan Analogues to Human Serum Albumin*

Rapier H. McMenamy and Richard H. Seder

From the Department of Biochemistry, State University of New York at Buffalo School of Medicine and the E. J. Meyer Memorial Hospital, Buffalo, New York

(Received for publication, November 6, 1962)

L-Tryptophan and some of its analogues were previously observed to bind predominately at one site on human serum albumin (1). The unique nature of this site which favors the binding of L-tryptophan with an association constant 100 times greater than n-tryptophan made it seem worthwhile to investigate the entropy, enthalpy, and free energy changes accompanying the association at this site. In addition to L-tryptophan, the other analogues used for this investigation were one without the ammonium group (3-indolepropionate), one without the carboxylate group (tryptamine), one with the charge removed from the ammonium group (acetyl-L-tryptophan), and one with neither the ammonium or carboxylate groups (skatole).

EXPERIMENTAL PROCEDURE

Albumin was obtained from the Protein Foundation as Fraction V (reworked), estimated to contain 97% albumin by electrophoretic analysis. Before use in the binding experiments the albumin was dissolved in water, dialyzed against several 100-fold volumes of a solution of 0.1 M NaCl containing 1 mM ethylenediaminetetraacetate, dialyzed against water, deionized by passing over a resin column as described by Dintzis (2), then dried from the frozen state in a vacuum. Solutions determined by refractometry to be from 0.36 to 0.43 mM (molecular weight taken as 69,000) were prepared from the dried powder. Albumin thus prepared was previously shown to bind L-tryptophan in the same manner as crystalline mercaptalbumin similarly deionized (1).

L-Tryptophan and the other analogues were obtained from Mann Research Laboratories, Inc. L-Tryptophan was recrystallized from 40% ethanol. The remainder of the reading.

The binding studies with L-tryptophan, tryptamine, acetyl-L-tryptophan, and skatole were conducted with equilibrium dialysis using a thin layer technique to hasten equilibrium (1). Analyzes of the dialysates for concentrations of the indole compounds were made by spectrophotometry (Beckman model DU, Beckman Instruments, Inc.) using an absorption maximum of 279 m\( \mu \), or in the case of skatole by spectrophotofluorometry (Aminco-Bowman spectrofluorometer, American Instrument Company, Inc.) at an excitation wave length of 280 m\( \mu \) and a fluorescence wave length of 375 m\( \mu \). One part of dialysate was diluted with 50 parts of 0.05 M phosphate buffer (pH 7.0) for the fluorometric assay.

The binding of indolepropionate was studied by centrifugal ultrafiltration (3), except for the competitive study, in which dialysis was used. Centrifugation was conducted for sufficient time to obtain 0.2 to 0.3 ml (10 to 20 minutes depending on the temperature). The content of indolepropionate in the ultrafiltrate was determined by spectrophotofluorometry (excitation wave length, 280 m\( \mu \); fluorescence wave length, 360 m\( \mu \)). A correction factor, as determined from ultrafiltrates in the absence of protein, was applied to the ultrafiltrate concentrations (Fig. 1). The correction involved was presumably due to differences in rates at which the indolepropionate and water pass through the membrane, and perhaps by a small amount of binding of indolepropionate to the membrane. Such rate differences and binding tendencies would not be apparent in dialysis studies in which equilibrium obtains and a much larger volume of solution is involved.

Paper chromatography was used for analysis in the competitive studies (1). Dialysate (3 ml) was dried from the frozen state. The residue was reconstituted by dissolving in 0.1 ml of Tergitol Nonionic NPX, Carbide and Carbon Chemicals Company. Standards were prepared by drying and reconstituting aliquots of similar salt content. Concentrations of skatole could not be determined by chromatography because of its volatility. Its value was obtained by taking a fluorometric measurement on the dialysate, subtracting the contribution of tryptophan as determined by paper chromatography from this measurement, and computing the skatole content from the remainder of the reading.

Binding studies were made in 0.1 M NaCl at different pH values with the protein used as a buffer. The temperatures were controlled to within 0.5 degree. The pH was measured at room temperature on completion of the binding experiment and adjusted by van't Hoff's equation to the temperature of the study, taking a \( \Delta H \) value for the adjustment from Tanford's data (4).

This correction factor is not peculiar to indolepropionate. Preliminary experiments indicate that a similar factor, showing a dependence on concentration, must be allowed for the correction of ultrafiltrate concentrations of other indole analogues, regardless of the charge they carry.
RESULTS

The data have been evaluated by the method previously described (1, 5). The relationship between the binding constants and number of binding sites is given by Equation 1

\[ \rho = \sum \frac{n_i k_i(A) \exp(2wZ_iZ_A)}{1 + k_i'(A) \exp(2wZ_iZ_A)} \]

where \( \rho \) denotes the unbound concentration of the small molecule; \( n_i \) is the number of sites with intrinsic association constant \( k_i' \) (the intrinsic constant here refers to the condition of binding in 0.1 M NaCl after correction has been made for electrostatic effects). The exponential term accounts for the electrostatic effect due to the charges on the molecules. The value of \( w \) was taken as 0.034 at 20° and 6°, and 0.035 at 37°. The net charge on the protein, \( Z_p \), was obtained from the electrometric titration of albumin as reported by Tanford (4), with an adjustment for chloride binding to the albumin. The number of Cl\(^-\) ions bound was estimated by extrapolation from the data of Scatchard et al. (6) (7 chloride ions bound at pH 7, 6 at pH 8, 5 at pH 9, 4 at pH 10). To facilitate determining \( k_i' \) and \( n_i \) for each set of sites the results are presented as plots of \( \rho/A \) versus \( \rho' \). By using Equation 1 and varying the values of \( k_i' \) and \( n_i \) a fit to the plotted data was obtained. In that there was only one primary site with an affinity much higher than the secondary sites the primary binding constant was estimated with fairly high accuracy.

The results of the binding studies are reported in Figs. 2 to 9, inclusive. Values for acetyl-L-tryptophan and L-tryptophan at 20° are taken from an earlier communication (1). That all compounds were bound to the same primary site was confirmed by competitive studies. Some of the competitive studies have been reported earlier (L-tryptophan and acetyl-L-tryptophan); others are shown in Figs. 10, 11, and 12. The binding constants computed for the primary site from competitive studies, although crude compared with those measured directly, leave little doubt that the same primary site is involved with all compounds. An interesting observation in the competitive studies was the enhancement of the binding of indolepropionate to secondary sites by the presence of L-tryptophan. This was evident from the cross-over of the binding curves in Fig. 11, a and b. This phenomenon was observed in three separate studies with two different albumin preparations. Such an enhancement in binding by the presence of a third molecule was previously noted by Karush with dyes (7).

The association constants obtained at two temperatures for the five compounds studied are listed in the second column of Table I. For indolepropionate, acetyl-L-tryptophan, and skatole, the constants are taken from the pH region 8 to 10, in which the constants did not vary with pH and were maximal. For L-tryptophan and tryptamine the association constants are obtained by fitting Equation 2 to plots of the observed association constants, \( k_i' \), at different pH values.

\[ k_i' = k_i^b \left[ \frac{(H^+)}{[H^+]} + K_p \exp\left(-2wZ_iZ_A\right)K_A + (H^+)K_p \right] \]

\[ + \left[ \frac{(H^+)}{[H^+]} + K_d \right] \]  

\( K_p \) is the apparent association constant for an acidic group postulated to be at the protein binding site. Evidence has previously indicated that the binding of L-tryptophan and its analogues are inhibited when both this acidic group on the protein, and the \( \alpha \)-amino group of L-tryptophan (or a similarly located amino group for other indole analogues) are in the acid forms (1). \( K_A \) is the apparent dissociation constant for the amino group on tryptophan (or tryptamine). For L-tryptophan, \( \rho K_A \) is 10.06 at 20° and 9.08 at 37°. For tryptamine, \( \rho K_A \) is 10.7.
**Fig. 3.** The binding of N-acetyl-L-tryptophan to human serum albumin at 37°, 0.1 M NaCl. O, pH 9.1; X, pH 8.9; A, pH 8.2; □, pH 7.4; ●, pH 6.4; ——, curve fitted for $n_1 = 1$, $k_1 = 6.7 \times 10^4$, $n_2 = 25$, $k_2 = 1.8 \times 10^6$ (values for pH 9.1-7.4); ———, curve fitted for $n_1 = 1$, $k_1 = 3.25 \times 10^4$, $n_2 = 25$, $k_2 = 1 \times 10^6$ (value for pH 6.4).

**Fig. 4.** The binding of tryptamine to human serum albumin at 2°, 0.1 M NaCl. No electrostatic correction was applied to the data. The pH at which the binding was conducted and the apparent association constant at the primary site ($\times 10^{-6}$) are indicated as follows: O, pH 10.5, $k_1 = 1.7$; X, pH 10.0, $k_1 = 1.55$; A, pH 9.3, $k_1 = 0.9$; □, pH 8.9, $k_1 = 0.5$; ●, pH 7.1, $k_1 < 0.1$.

**Fig. 5.** The binding of tryptamine to human serum albumin at 37°, 0.1 M NaCl. No electrostatic correction has been applied to the data. The pH at which the binding was conducted and the apparent association constant at the primary site ($\times 10^{-6}$) are indicated as follows: O, pH 9.75, $k_1 = 1.0$; X, pH 9.5, $k_1 < 0.9$; △, pH 9.3, $k_1 = 0.31$; □, pH 7.7, $k_1 = 0.07$; ●, pH 6.7, $k_1 < 0.01$.

**Fig. 6.** The binding of indole-3-propionate to human serum albumin at 6°, 0.1 M NaCl. O, pH 9.8; X, pH 9.4; A, pH 8.9; □, pH 7.4; ——, curve fitted for $n_1 = 1$, $k_1 = 5 \times 10^5$, $n_1 = 1$, $k_1 = 2 \times 10^5$, $n_1 = 1$, $k_1 = 1 \times 10^6$ (values for pH 9.8 to 8.9); ———, curve fitted for $n_1 = 1$, $k_1 = 1.8 \times 10^4$, $n_1 = 1$, $k_1 = 4 \times 10^5$, $n_1 = 1$, $k_1 = 2 \times 10^5$ (values for pH 7.4).
The binding of indole-3-propionate to human serum albumin at 36° to 0.1 m NaCl. O, pH 9.0; X, pH 8.7; △, pH 8.2; □, pH 7.1; ---, curve fitted for $n_1 = 1$, $k_1 = 2.5 \times 10^6$, $n_2 = 1$, $k_2 = 1 \times 10^4$, $n_3 = 1$, $k_3 = 5 \times 10^4$ (values for pH 9.0 to 8.2); -- - , curve fitted for $n_1 = 1$, $k_1 = 1.0 \times 10^6$, $n_2 = 1$, $k_2 = 3 \times 10^4$, $n_3 = 1$, $k_3 = 1.5 \times 10^4$ (values for pH 7.1).

The value of L-tryptophan was obtained from a titration study at 2° (1). Its value at different temperatures was obtained from van’t Hoff's equation assuming $\Delta H = -10.0$ kcal. The dissociation constants for tryptamine were determined from titrations at 10° and 37°. The resultant $\Delta H = -8.8$ kcal was used to adjust the $pK$ to 2°.

No electrostatic correction was applied. O, Binding of L-tryptophan in absence of tryptamine; ●, binding of L-tryptophan in the presence of 2.54 moles of tryptamine per mole of albumin; $k'_1$ for L-tryptophan, $2.5 \times 10^4$; computed $k'_1$ for tryptamine, $0.5 \times 10^4$.
FIG. 11. The competitive binding of L-tryptophan and indolepropionate to human serum albumin at pH 7.8, 0.1 M NaCl, 0.01 M ethylenediaminetetraacetate. No electrostatic correction was applied. 0, Binding of indolepropionate in the absence of L-tryptophan; O, binding of indolepropionate in the presence of approximately 1 mole of tryptophan per mole of albumin. a, Study at 37°; $k'_i$ indolepropionate, 0.8 X 10°; computed $k'_i$ for L-tryptophan, 1.6 X 10°. b, Study at 15°; $k'_i$ indolepropionate, 1.5 X 10°; computed $k'_i$ for L-tryptophan, 7.5 X 10°.

FIG. 12. The competitive binding of skatole and L-tryptophan to human serum albumin at 18°, 0.1 M NaCl and 0.01 M ethylenediaminetetraacetate, pH 8.35. No electrostatic correction was applied. O, Binding of skatole alone; O, binding of skatole in presence of 0.667 mole of L-tryptophan per mole of albumin.

TABLE I

Thermodynamic changes in association of indole analogues to human serum albumin*

<table>
<thead>
<tr>
<th>Compound</th>
<th>$T$</th>
<th>$k' \times 10^{-4}$</th>
<th>$\Delta F^\circ$</th>
<th>$\Delta H^\circ$</th>
<th>$\Delta S^\circ$</th>
<th>$\Delta F_u$</th>
<th>$\Delta S_u$</th>
<th>$\Delta F_{d-o}$</th>
<th>$\Delta S_{d-o}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skatole</td>
<td>290</td>
<td>5.5</td>
<td>-6.3</td>
<td>-6.0</td>
<td>-10.5</td>
<td>-8.5</td>
<td>-7</td>
<td>0</td>
<td>-8.5</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>1.7</td>
<td>-6.0</td>
<td>-10.5</td>
<td>-15</td>
<td>-8.5</td>
<td>-7</td>
<td>0</td>
<td>-8.5</td>
</tr>
<tr>
<td>Indoleethylene group</td>
<td>298</td>
<td></td>
<td>-7.2</td>
<td>-12.5</td>
<td>-18</td>
<td>-9.6</td>
<td>-10</td>
<td>-3</td>
<td>-10.4</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>275</td>
<td>0.067</td>
<td>-3.5</td>
<td>-2.0</td>
<td>6</td>
<td>-6.0</td>
<td>14</td>
<td>-6</td>
<td>-8.0</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>0.044</td>
<td>-3.7</td>
<td>-2.0</td>
<td>6</td>
<td>-6.0</td>
<td>14</td>
<td>-6</td>
<td>-8.0</td>
</tr>
<tr>
<td>Indolepropionate</td>
<td>279</td>
<td>500.0</td>
<td>-8.5</td>
<td>-4.0</td>
<td>16</td>
<td>-11.3</td>
<td>24</td>
<td>-6</td>
<td>-12.9</td>
</tr>
<tr>
<td></td>
<td>309</td>
<td>250.0</td>
<td>-9.0</td>
<td>-4.0</td>
<td>16</td>
<td>-11.3</td>
<td>24</td>
<td>-6</td>
<td>-12.9</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>275</td>
<td>13.0</td>
<td>-6.4</td>
<td>-2.3</td>
<td>15</td>
<td>-9.1</td>
<td>23</td>
<td>-8</td>
<td>-11.5</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>8.0</td>
<td>-7.0</td>
<td>-2.3</td>
<td>15</td>
<td>-9.1</td>
<td>23</td>
<td>-8</td>
<td>-11.5</td>
</tr>
<tr>
<td>Acetyl-L-tryptophan</td>
<td>275</td>
<td>19.0</td>
<td>-6.6</td>
<td>-5.0</td>
<td>6</td>
<td>-9.1</td>
<td>14</td>
<td>-13</td>
<td>-13.0</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>6.75</td>
<td>-6.8</td>
<td>-5.0</td>
<td>6</td>
<td>-9.1</td>
<td>14</td>
<td>-13</td>
<td>-13.0</td>
</tr>
</tbody>
</table>

* $\Delta F$ and $\Delta H$ are expressed in kilocalories per mole; $\Delta S$ is in entropy units (calories per mole per degree).

8.3). The fitted curves allow for little variability in the intrinsic association constants.

The standard molar changes, free energy ($\Delta F^\circ$), enthalpy ($\Delta H^\circ$), and entropy ($\Delta S^\circ$), for the association at the primary site of each compound and the indoleethylene group (a common stem of the indole analogues) are reported in Table I. The values for the compounds were obtained from Equations 3 and 4 where it is assumed that $\Delta S^\circ$ and $\Delta H^\circ$ are constant in the temperature range studied.

$$\Delta F^\circ = -RT \ln k^4 \quad (3)$$

$$\Delta S^\circ = (\Delta H^\circ - \Delta F^\circ)/T \quad (4)$$

$\Delta F^\circ$ is accurate to within 0.1 kcal; $\Delta H^\circ$ to within 1.0 kcal; and $\Delta S^\circ$ to within 3 entropy units (e.u.). Values for the indoleethylene stem were obtained by adding to the skatole values an extra energy increment to approximate that of an additional CH$_2$ group; -1.1 kcal for $\Delta F$, -2.0 kcal for $\Delta H$, and -3.0 e.u. for $\Delta S$ (the contribution of one hydrogen was neglected).

The thermodynamic parameters obtained here ($\Delta S^\circ$, $\Delta H^\circ$, $\Delta F^\circ$) are expressed in kilocalories per mole; $\Delta S$ is in entropy units (calories per mole per degree).

The difference between the binding of skatole and indole (which has one CH$_2$ group less) was -1.1 kcal in an unreported experiment. This value is in good agreement with the value of -1.1 kcal estimated by Waugh (10) for the van der Waal's association energy of the CH$_2$ group. $\Delta S$ is computed as an extra segment added to skatole by Flory's equation. This leads to a $\Delta H$ of -2.0 kcal per mole at 25° which is reasonable in view of a computed maximal value of -2.6 kcal per mole by other means (11).
Fig. 13. The pH dependence of L-tryptophan binding to human serum albumin, 0.1 M NaCl. •, Studies at 37°; ---, fitted curve by Equation 2, \( k^0 = 8 \times 10^4 \); ---, curve from previous study at 2° (1); \( k^0 = 13 \times 10^4 \).

Fig. 14. The pH dependence of tryptamine binding to human serum albumin, 0.1 M NaCl. O, Studies at 2°; \( k^0 = 670 \); •, studies at 37°; \( k^0 = 440 \). Curves fitted by Equation 2.

are those describing the conversion of the solution state of the molecule to the state of associated complex. A cratic entropy term (−8 e.u.) can therefore be subtracted from the association entropies of each compound to obtain unitary values, \( \Delta S^u \), \( \Delta S^a \), and \( \Delta S^u - d \) (8, 9). These unitary values are reported in Columns 6 and 7, Table I. The molar entropy changes due to loss in disorientational freedom of the small molecules as they associate to the protein varies from compound to compound depending upon the number of segments, \( x \), i.e., the units which can freely rotate with respect to one another, in the compounds. To place the thermodynamic values of the compounds with multiple groups on a comparable basis the molar entropy changes due to the loss in disorientational freedom by the small molecules as they associate with the protein must be taken into account. The disorientational entropy losses (\( \Delta S_d \)) have been estimated by Flory's equation (12). (The sign is reversed for the association process.)

\[
\Delta S_d = R [\ln x + (x - 1) \ln ((x - 1)/6)]
\]

\( x \) is 2 for the indolylethylene group, 3 for tryptamine, 3 for indolepropionate, 4 for L-tryptophan, and 6 for acetyl-L-tryptophan; and \( z \) (the lattice coordination number) is taken as 8. Flory's equation is based upon a number of approximations, i.e., the segment size is taken to be the same as that of the solvent molecules, the lattice number is chosen empirically, complete randomness of the segments is assumed when the molecule is in solution, and the loss of all segment freedom is assumed when the molecule is associated in the protein complex. It has, however, been found to give entropy values of approximately the right magnitude for the segment disorientation of a number of different polymers; even for segments containing groups of different type and size. (For example, values of 1.5 to 2 e.u. per segment were found in the fusion of polymers with segments varying in size from \(-\text{CH}_2-\) to \(-\text{CO}-\), compared with average calculated values of approximately 2 for polymer segments by Flory's equation (12). The unitary values less \( \Delta S_d \), designated \( \Delta S_a - d \), are reported for the compounds in Table I. \( \Delta S_a - d \) on the basis of the above considerations is the entropy change external to the associated small molecule. It should relate principally to the change in the protein and the solvent structure at the site of the association.

Taking the association energies as separable and additive for the different groups of the ligand, \( \Delta F_a \), \( \Delta S_a \), and \( \Delta F_a - d \) are obtained for the ammonium group, the carboxylate group, the ammonium-carboxylate groups, and the acetamide-carboxylate groups by subtracting the value of the indolylethylene stem from the appropriate compound values in Table I. These group values are reported in Table II.

**DISCUSSION**

**Association Energies of Compounds**

Except for skatole, the entropy changes found for the association of the indole analogues to serum albumin (Table I) are in the same general range as the values found for nonindole compounds. \( \Delta S_a \) was found to vary from 10 to 40 e.u. for a number of other small organic compounds (9), which compares favorably with...
\[ \Delta S_a \] for the compounds studied here, omitting skatole. \[ \Delta S_a \] for the association of skatole is \(-7\) e.u. This is atypical, and to our knowledge no other small compound has been observed to bind to serum albumin with an entropy loss of this magnitude. \[ \Delta H_a \] for skatole is also low compared with values for other compounds; \(-10.5\) kcal per mole compared with a value of \(-2\) to \(-5\) kcal per mole for the other compounds in Table I, or 0 to \(-4\) kcal per mole compared with the values for the small organic compounds listed by Kauzmann (9).

**Association Energies of Different Groups**

The indolylethylene group supplies the major part of the energy for the association of the small molecules to the protein site, as a \[ \Delta F_a \] of \(-8.5\) kcal per mole attests. Since \[ \Delta S_a \] (as well as \[ \Delta S_{a-d} \]) is negative, the energy of binding of the indolylethylene group is derived from the large negative enthalpy term (\(-12.5\) kcal per mole). The value for \[ \Delta H^\circ \] is well under the maximum of \(-20.0\) kcal per mole estimated to be available for the association of this group from vapor pressure measurements.

On the other hand, one would expect a positive entropy of the order of 15 to 20 e.u. for the removal of the indolylethylene group from water; compare, for example, with the reverse process of adding benzene, toluene, or xylene to water in which \[ \Delta S_a \] of \(-14\) to \(-20\) e.u. has been observed (9). Subtracting this value from \[ \Delta S_a \] (\(-10\) e.u.) and \[ \Delta S_{a-d} \] (\(-8\) e.u.) leaves an entropy change of \(-30\) to \(-40\) e.u. assigned to the formation of the protein-indolylethylene group complex. It is probable that this large negative entropy change is responsible for the well known stabilizing property of compounds such as acetyl-l-tryptophan (which contains the indolylethylene group) against the thermal denaturation of albumin. The arrest of torsional movements of key hydrophobic groups on the albumin by the associated ligand would explain the increase in the thermal energy required to initiate the denaturation process.

The free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]). The positive values indicating that the association is not favored by the free energy contributed by the charged amino group in complex formation with the protein, as determined by the difference between the binding of the tryptamine and the indolylethylene group, is \(-3.6\) kcal per mole (\[ \Delta F_a \]), or after allowance for disorientation losses in the ligand, \(-2.4\) kcal per mole (\[ \Delta F_{a-d} \]).
the displacement of more water from the groups than in the case of one point contact as with simple aquo-ion pairing.

**SUMMARY**

The binding of L-tryptophan, indolepropionate, tryptamine, acetyl-L-tryptophan, and skatole to human serum albumin has been studied by equilibrium dialysis or ultrafiltration at two temperatures, and the free energy (ΔF°), enthalpy (ΔH°), and entropy (ΔS°) changes for the associations have been obtained. From the differences in the thermodynamic values for these indole analogues, unitary values, ΔF°, ΔH°, ΔS°, can be obtained for the different groups. In the order of ΔF° (kilocalories per mole), ΔH° (kilocalories per mole), ΔS° (entropy units), the values for these groups are as follows: indolylethylene, −8.5, −12.5, −10; NH₄⁺, 3.6, 10.4, 24; −COO⁻, −1.7, 8.5, 34; NH₃⁺−COO⁻, 0.5, 10.1, 33; CH₃CONH−COO⁻, 0.5, 7.4, 24. Comparison between the groups after allowance for disorientation entropy losses in the association of the indole analogues have also been made. In the association of the indolylethylene group, −30 to −40 e.u. are attributed to entropy changes at the protein site, presumably due to the loss in freedom of hydrophobic groups on the protein. Enthalpy changes are favorable to the association of this group. For the other groups, which are hydrophilic in nature, the entropy change, not the enthalpy change, accounts for the energy favorable to the association.

**Acknowledgment**—Appreciation is expressed to Drs. Margaret Hunter and J. L. Oncley for the opportunity to discuss with them various parts of this manuscript.

**REFERENCES**

Thermodynamic Values Related to the Association of L-Tryptophan Analogues to Human Serum Albumin
Rapier H. McMenamy and Richard H. Seder


Access the most updated version of this article at http://www.jbc.org/content/238/10/3241.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/238/10/3241.citation.full.html#ref-list-1