Biochemistry **1992,** *31,* 12813–12818

Partitioning of Tryptophan Side-Chain Analogs between Water and Cyclohexane†

William C. Wimley and Stephen H. White*

Department of Physiology and Biophysics, University of California, Irvine, California, 92717

Received July 10, 1992; Revised Manuscript Received October 13, 1992

ABSTRACT: We have measured the partitioning of the tryptophan side-chain analogs 3-methylindole and *N*-methylindole between water and cyclohexane over the temperature range 8–55 °C to investigate the relative contribution of the imine -NH- to the free energy of transfer. We take advantage of the fact that the indole imine nitrogen is blocked by a methyl group in *N*-methylindole. Unlike previous studies, we take into account the water present in the cyclohexane phase. Free energies of partitioning were calculated using mole-fraction, volume-fraction, and Flory–Huggins-corrected volume-fraction partition coefficients [De Young, L. R., & Dill, K. A. (1990) *J. Phys. Chem. 94*, 801–809; Sharp, K. A., Nicholls, A., Friedman, R., & Honig, B. (1991) *Biochemistry 30*, 9686–9697]. These approaches account for configurational entropy changes in different ways and thus lead to different values for the calculated free energies of transfer. There is a 2–3-fold difference in the free energies calculated from our measurements, using the different units. Independent of units, the partitioning of both compounds involves identical entropy changes. However, 3-methylindole has an additional unfavorable enthalpic contribution to partitioning into cyclohexane of +1.6 kcal/mol (independent of units) which is presumably the cost of removing the indole -NH- group from water and transferring it to cyclohexane. In cyclohexane, 3-methylindole forms hydrogen bonds with water that cause water to copartition into cyclohexane with the solute. A method is described which allows the partitioning process to be examined independent of subsequent interactions with water in the solvent.

We present data for the partitioning of the tryptophan side chain analogs 3-methylindole (3MI)[1] and *N*-methylindole (NMI) between water and cyclohexane. These two compounds are isomers that differ only by the location of the methyl group; 3MI is identical to the side chain of tryptophan with a free imine -NH- group which can form hydrogen bonds, while NMI has the nitrogen blocked with the methyl group. The purpose of these experiments was to examine (1) the contribution of the -NH- group to the free energy of partitioning, (2) the effect of interactions of the solutes with water in cyclohexane, and (3) the effect of using different partition coefficient units on the free energy of partitioning.

Among the various hydrophobicity scales which rank the naturally occurring amino acids, tryptophan is the most variable. It ranks as the most hydrophobic on the basis of its partitioning into polar solvents like octanol (Fauchere & Pliska, 1983), while scales based on partitioning into nonpolar solvents such as cyclohexane (Radzicka & Wolfendon, 1988) rank tryptophan as only intermediate in hydrophobicity. This ambiguity results from the fact that while tryptophan has the polar -NH- group which is capable of forming hydrogen bonds, it also has the largest nonpolar accessible surface area of the natural amino acids. The relative hydrophobicity depends on whether the -NH- hydrogen bond can be satisfied in the solvent phase. The free energy cost of removing the tryptophan -NH- group from water to a nonpolar environment has been estimated to be between 3.0 kcal/mol (Engelman & Steitz, 1981) and 4.1 kcal/mol (Jacobs & White, 1989). In the work presented here, we have measured this value and find that it is 1.6 kcal/mol after correction for the interaction of 3MI with water in cyclohexane. Therefore, the indole -NH- group of tryptophan is significantly less polar than previously estimated.

The assumption is often made that solvents such as cyclohexane have negligible water contents and, as such, constitute truly nonpolar reference phases. In fact, however, the solubility of water in all common nonpolar solvents is between 2 and 4 mM (Przybytec, 1980) and there may, therefore, be no solvent that constitutes a truly nonpolar reference phase in a water-to-solvent partitioning experiment. Thus, polar solutes such as 3MI may interact with the water dissolved in the solvent. If the interaction between a polar solute and water dissolved in the solvent phase is ignored, then the water-to-solvent partition coefficient will be overestimated and the molecule will appear to be less polar. We have studied the interactions between 3MI, NMI, and water in cyclohexane and find that an interaction between 3MI and water occurs which leads to an equilibrium between hydrated and unhydrated 3MI in cyclohexane. The two forms are approximately equally populated over the temperature range studied. The free energy of partitioning can be calculated independent of subsequent interactions with water in the solvent phase because the concentrations of the hydrated and unhydrated forms in the solvent are known. If the total concentration of 3MI in cyclohexane is used to calculate the free energy, instead of the unhydrated form only, the partition coefficient will be overestimated by a factor of 2 causing the free energy of transfer to be overestimated by 0.4 kcal/mol.

Free energies of partitioning are calculated from partition coefficients by

$$\Delta G = -RT \ln (K_p) \tag{1}$$

where R is the gas constant, T is the absolute temperature, and K_p is the partition coefficient. The right side of eq 1 is the ideal mixing entropy. A problem that arises in such calculations is the choice of the units of the partition coefficient which is equivalent to defining the standard state. Most

---

† Supported in part by NSF and NIH. W.C.W. is supported by U.S. PHS Grant GM14178.

* To whom correspondence should be addressed.

[1] Abbreviations: 3MI, 3-methylindole; NMI, *N*-methylindole; HPLC, high-pressure liquid chromatography.

0006-2960/92/0431-12813$03.00/0

commonly, mole-fraction units have been used to determine amino acid hydrophobicity scales (Nozaki & Tanford, 1971; Radzicka & Wolfendon, 1988) and to estimate the hydrophobic-effect contribution to protein folding (Baldwin, 1986; Privalov & Gill, 1988; Murphy et al., 1990), but volume-fraction partition coefficients have also been used [e.g., Ben-Naim and Wilf (1979) and Simon et al. (1982)]. However, Flory (1941) and Huggins (1941) showed, using polymer theory, that the ideal mixing entropy term (eq 1) used to calculate mole-fraction- or volume-fraction-based free energies is only strictly correct when the solute and solvents have the same molar volumes. Because water has a low molar volume, this is rarely the case.

The different systems of units account for the configurational entropy of partitioning in different ways and thus lead to different values for ΔG in eq 1. Sharp et al. (1991) have discussed this problem in detail and derived an expression for the free energy of partitioning of solutes between solvents using ideal gas theory that takes into account the configurational entropy change associated with the transfer of a solute between solvents with different molar volumes. This expression is identical to that obtained by Flory (1941) and Huggins (1941):

$$\Delta G_{\mathrm{a-b}} = -RT\ln(K_{\mathrm{v}}) + RT\left(\frac{V_{\mathrm{s}}}{V_{\mathrm{b}}} - \frac{V_{\mathrm{s}}}{V_{\mathrm{a}}}\right) \qquad (2)$$

where $\Delta G_{\mathrm{a-b}}$ is the free energy of transfer of a solute from solvent a to solvent b and K_{v} is the volume-fraction partition coefficient. V_{s}, V_{a}, and V_{b} are, respectively, the molar volumes of the solute, solvent a, and solvent b. In the absence of experimentally determined partial molar volumes, one assumes that they are equal to the molar volumes of the pure components. In that case, K_{v} is the ratio of the molar concentrations of the solute in solvent b to solvent a. Consensus on the best scale to use for partitioning has not yet emerged. However, De Young and Dill (1990) have shown that the partitioning of benzene between water and *n*-alkane solvents ($n = 8, 10, 12, 14, 16$) is best described by the Flory–Huggins formulation. In addition, Sharp et al. (1991) have shown that the partitioning of *n*-alkanes from the gas phase into water scales uniformly with chain length only using the Flory–Huggins approach. Because of the lack of consensus, we will use all three sets of units to calculate the free energies of 3MI and NMI partitioning in order to examine the effects of different choices on the results.

MATERIALS AND METHODS

Chemicals. *N*-Methylindole and 3-methylindole were obtained from Sigma (St. Louis, MO) and were found to be greater than 99.5% pure using high-pressure liquid chromatography (HPLC). HPLC-grade cyclohexane was obtained from Fisher (Fair Lawn, NJ) or from Burdick and Jackson (Muskegon, MI). Water was glass distilled. Tritiated water was obtained from New England Nuclear (Boston, MA) and diluted with unlabeled water to give a specific activity of 4.93 $\times 10^9$ dpm/mol of H_2O. The mole ratio of tritium to hydrogen is 10^{-7}.

Water Content of Cyclohexane. Water content was determined using a Beckman Model LS 7000 liquid scintillation counter and Fisher Scintiverse BD scintillation cocktail. Samples contained 10^3–10^4 dpm and were counted for 10 min each. The specific activity of the ^3H_2O was measured using volumes equivalent to between 0.2 and 200 nL of the stock solution; all gave the same value. In the experiments, 1 mL of cyclohexane containing between 0 and 25 mM of *N*-methylindole or 3-methylindole was mixed with 2–5 μL of ^3H_2O. The solutions were immersed in a temperature-controlled water bath and were rotated about the long axis of the vial at 10 rpm. After overnight equilibration, aliquots of 100 μL were removed and added to 15 mL of scintillation cocktail and the ^3H_2O was measured by liquid scintillation counting. All samples still had visible amounts of excess water after equilibration. Samples of the stock ^3H_2O, in the presence of 100 μL of cyclohexane, were also counted at the same time.

Indole Partitioning Experiments. Concentrated methanol solutions of 10–25 mM NMI or 3MI were used to prepare 50 μM cyclohexane solutions and 5 μM (3MI) or 1 μM (NMI) solutions in water. Equal 1–2-mL volumes of the water and cyclohexane solutions were mixed together in 4-mL amber vials. The samples were immersed in a temperature-controlled circulating water bath and rotated about the long axis of the vial at approximately 10 rpm. After overnight equilibration, the two phases were carefully separated and allowed to equilibrate at room temperature for at least 1 h. Samples of the stock water and cyclohexane solutions were treated identically. Prior to equilibration, 10 μL of water was added to the stock cyclohexane solutions to ensure saturation of the solutions. After several high-temperature experiments, the probe purity was reexamined by HPLC. No breakdown of the compounds was observed.

Indole concentrations were determined by measuring the fluorescence of the solutions and comparing the values to those obtained from the stock solutions in water or cyclohexane. A SPEX Fluorolog fluorescence spectrophotometer was used which has been upgraded and interfaced to a computer by OLIS instrument systems (Jefferson, GA). The excitation maximum for all samples was 288 nm. Emission was at 375 nm for 3-methylindole in water, 365 for *N*-methylindole in water, 325 for 3-methylindole in water-saturated cyclohexane, and 320 for *N*-methylindole in water-saturated cyclohexane. Slits were set at 10 nm. Linearity of response was demonstrated in the range 0.1–50 μM probe in both water and cyclohexane. The sum of the concentrations measured in the experiments was always found to be equal to the sum of the starting concentrations within experimental error. To assure that the methanol did not affect the experimental results, we conducted a set of experiments over the temperature range 15–55 °C in which solutions of 3-methylindole in water and in cyclohexane were made directly with solid 3-methylindole. The results from these experiments were identical to those obtained from methanol-containing experiments.

Calculation of Free Energies. The free energies of partitioning were calculated using mole-fraction, volume-fraction, and Flory–Huggins-corrected volume-fraction partition coefficients (eq 2). Molar volumes of pure compounds were calculated from molecular weights and from density data found in the *TRC Thermodynamic Tables* (1990) or in the *CRC Handbook of Chemistry and Physics* (Weast, 1981).

The calculation of volume-fraction or Flory-Huggins-corrected volume-fraction partition coefficients requires that one know the partial molar volume of the solute in both solvents. If mixing is ideal, this will be equal to the molar volume of the pure substance. The molar volume of pure *N*-methylindole (liquid at room temperature) is 0.126 L/mol at 25 °C, but 3-methylindole is a solid at room temperature. We assumed that, in the very dilute solutions used in these experiments, the partial molar volume of 3MI is the same as that of NMI. Benzene and toluene have partial molar volumes in water which are respectively factors of 1.07 and 1.11 times larger than the molar volumes of the pure compounds at 25 °C

(Makhatadze & Privalov, 1988). We estimate that the molar volumes of 3MI and NMI in water are a factor of 1.1 larger than the molar volume of pure NMI at 25 °C. The partial molar volumes in cyclohexane are assumed to be equal to the molar volume of pure NMI. A 5% error in the partial molar volumes will result in an error in the Flory–Huggins-based free energy of approximately ±0.15–0.20 kcal/mol. This uncertainty is much larger than that derived from the uncertainty in the experimentally measured parameters which is ±0.02–0.03 kcal/mol. We assume that the temperature dependence of the partial molar volume of NMI and 3MI is the same as that of pure NMI. The contribution of the uncertainty in the temperature dependence of the partial molar volumes to the uncertainty in the calculated free energy of partitioning is small compared to the contribution of the uncertainty in the absolute partial molar volumes at 25 °C.

All concentrations were measured at 25 °C, but this will not be the equal to the equilibrium concentration at other temperatures. This necessitates a correction to the measured concentration:

$$[\mathrm{S}](T) = [\mathrm{S}](298)\left(\frac{V(298)}{V(T)}\right) \tag{3}$$

where the $[\mathrm{S}](T)$ is the molar concentration of the solute at temperature T, $[\mathrm{S}](298)$ is the measured concentration, and $V(298)$ and $V(T)$ are the molar volumes of the solvent at T = 298 °C and at the experimental temperature, respectively.

Copartitioning of Water into Nonpolar Solvents. When a partially polar solute partitions between water and a solvent, there is the possibility that the solute can interact with the water dissolved in the solvent. The solubility of water in nonpolar solvents is typically 2–4 mM (Przybytec, 1980), a nonnegligible amount in many experiments. Solute or water partitioning between water and a solvent can be described with a simple equilibrium $[\mathrm{S}]_\mathrm{w} \leftrightarrow [\mathrm{S}]_\mathrm{slv}$ so that

$$K_\mathrm{p} = \frac{[\mathrm{S}]_\mathrm{slv}}{[\mathrm{S}]_\mathrm{w}} \tag{4}$$

where [S] indicates the concentration (or mole fraction) of solute or water, the subscripts w and slv indicate the water phase or solvent phase concentrations, respectively, and K_p is the equilibrium constant. An additional equilibrium occurs when a solute interacts with a water molecule in the solvent: $[\mathrm{W}]_\mathrm{slv} + [\mathrm{S}]_\mathrm{slv} \leftrightarrow [\mathrm{SW}]_\mathrm{slv}$ so that

$$K_\mathrm{h} = \frac{[\mathrm{SW}]}{[\mathrm{S}][\mathrm{W}]} \tag{5}$$

where [S] is the concentration (or mole fraction) of solute in the solvent, [SW] is the concentration of the "hydrated" solute species, [W] is the concentration of free water in the solvent, and K_h is the equilibrium constant for the interaction with water. The remainder of the equations in this section deal with concentrations in the solvent phase only, and the subscript slv has therefore been dropped. One must know these equilibrium constants in order to separate the thermodynamics of partitioning from the interaction with water in the solvent.

This information can be obtained in a separate experiment. A solvent containing various concentrations of solute is equilibrated with a very small amount of excess labeled water, such that the partitioning of the solute from the solvent into the water is negligible. The total amount of water in the solvent

$$[\mathrm{W}]_\mathrm{tot} = [\mathrm{W}] + [\mathrm{SW}] \tag{6}$$

is measured and combined with eq 5 above gives

$$[\mathrm{W}]_\mathrm{tot} = [\mathrm{W}] + [\mathrm{S}]_\mathrm{tot}\left(\frac{K_\mathrm{h}[\mathrm{W}]}{1 + K_\mathrm{h}[\mathrm{W}]}\right) \tag{7}$$

where $[\mathrm{W}]_\mathrm{tot}$ and [W] are the concentrations (or mole fractions) of total water and free water in the solvent, respectively, $[\mathrm{S}]_\mathrm{tot}$ is the total concentration (or mole fraction) of solute, and K_h is the equilibrium constant that describes the interaction of the solute polar groups with water in the solvent (eq 5). K_h is determined from the dependence of the total water content on solute concentration.

The concentration of unhydrated solute in the solvent [S] can be calculated from K_h by

$$[\mathrm{S}] = \left(\frac{[\mathrm{S}]_\mathrm{tot}}{1 + K_\mathrm{h}[\mathrm{W}]}\right) \tag{8}$$

It is the concentration of unhydrated solute in the solvent that should be used in the calculation of the free energy of partitioning of a solute into a solvent. Free energy calculations based on the total concentration of the solute in the solvent will be erroneous and will overestimate the water to solvent partition coefficient. The magnitude of the error will be

$$\Delta\Delta G = -RT \ln\left(\frac{[\mathrm{S}]}{[\mathrm{S}]_\mathrm{tot}}\right) \tag{9}$$

As discussed below, we have observed an interaction between 3MI and water in cyclohexane and have measured the hydration equilibrium constant for this interaction.

RESULTS AND DISCUSSION

Indole Partitioning. In a partitioning experiment, the interaction between partially polar solutes and water dissolved in the solvent phase must be accounted for in the calculation of partition coefficients. Consequently, we measured the concentration of water in water-saturated cyclohexane as a function of temperature and NMI or 3MI concentration. The results are shown in Figure 1A,B. The concentration of water in water-saturated cyclohexane is 2.4 ± 0.1 mM at 25 °C and increases to 4.4 ± 0.4 mM at 55 °C (see Figure 1A). Figure 1B shows the changes in the water content of cyclohexane with 3MI concentration. The addition of up to 25 mM NMI had no effect on the water content (data not shown). On the other hand, 3MI significantly increased the amount of dissolved water, indicating an interaction between 3MI and water in cyclohexane. The slope, averaged over all temperatures, is 0.49 ± 0.05 mol of H_2O/mol of 3MI. This slope suggests that 3MI exists as a water-bridged dimer in cyclohexane. This is unlikely, however, because of the prohibitive steric (i.e., entropic) cost of hydrogen bonding two large double ring molecules to a single oxygen atom separated by an angle of approximately 110° (Janin & Chothia, 1978; Erickson, 1978). These two indoles have the same accessible nonpolar surface area, and thus should have the same hydrophobic-effect entropy driving the partitioning (Tanford, 1980). As discussed below, we find that the entropies driving the partitioning of 3MI and NMI are the same. Therefore, there are no additional unfavorable entropy contributions so that the slope of 0.5 indicates that hydrated and unhydrated 3MI are approximately equally populated in cyclohexane over the temperature range studied. The temperature dependence of the free energy of 3MI hydration in cyclohexane (eq 1) indicates that this process is driven by enthalpy changes.

We have assumed that each *hydrated* 3MI molecule interacts with a single water. This is reasonable since only

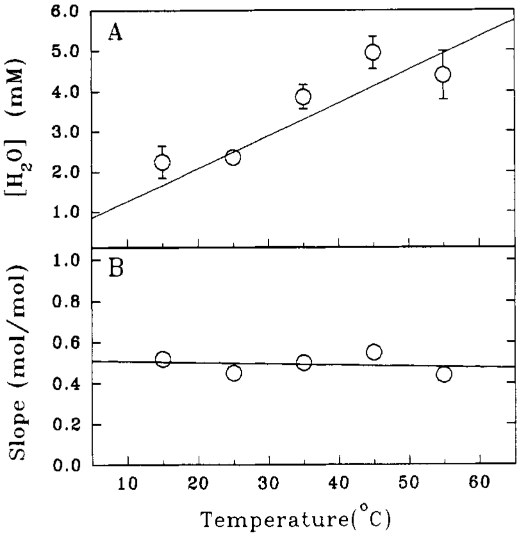

FIGURE 1: Water content of cyclohexane. (A) Concentration of water in water-saturated cyclohexane in the absence of 3MI or NMI. Water concentration was measured with ^3H_2O as described in the text. Ten experiments were done at 25 °C, and one or two were done at all other temperatures. (B) Change in water content of cyclohexane with 3MI. Solutions of 0–25 mM 3MI in cyclohexane were equilibrated with 2–5 μL of ^3H_2O. All experiments showed a linear increase in water content with 3MI concentration. Panel B shows the slopes of the lines in moles of H_2O per mole of 3MI. Uncertainties were estimated from the scatter in the data (Bevington, 1969) and are approximately equal to the symbol size.

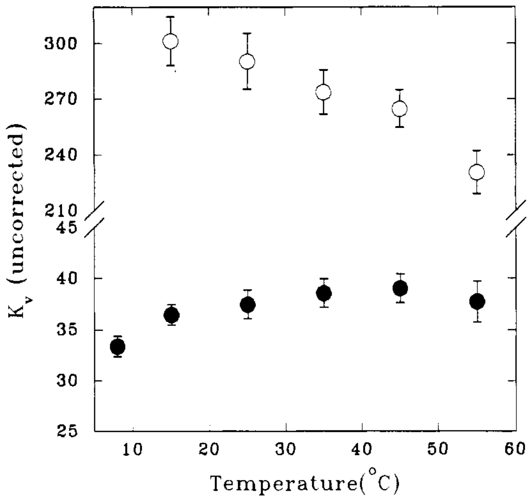

FIGURE 2: Uncorrected experimental data for 3MI and NMI partitioning. Filled circles represent 3MI data, and open circles represent NMI data. K_v is the uncorrected volume-fraction partition coefficient which is equal to the ratio of the molar concentration of 3MI or NMI in cyclohexane to that in water. The 3MI data have not been corrected for hydration in cyclohexane (see text). Each point is the average of 5–15 experiments, and the uncertainties are estimated from the scatter in the individual values.

Table I: Experimental Data for Water–Cyclohexane Partitioning of 3-Methylindole and *N*-Methylindole

temperature (K)	K_v	ΔG^a VF	MF	FH
	N-Methylindole			
288	301 ± 13[b]	−3.21	−4.29	−6.88
298	290 ± 15	−3.30	−4.42	−7.15
308	274 ± 12	−3.37	−4.54	−7.42
318	265 ± 10	−3.46	−4.67	−7.69
328	231 ± 12	−3.48	−4.73	−7.87
	3-Methylindole			
281	17.0 ± 0.5	−1.53	−2.57	−5.07
288	18.6 ± 0.5	−1.62	−2.69	−5.28
298	19.1 ± 0.7	−1.69	−2.81	−5.54
308	19.7 ± 0.7	−1.76	−2.93	−5.81
318	19.9 ± 0.7	−1.83	−3.03	−6.06
328	19.2 ± 1.0	−1.86	−3.11	−6.28

[a] Free energies are in kilocalories per mole and are for the partitioning from water into cyclohexane. Calculation of free energies is described in the text. [b] K_v is the volume-fraction partition coefficient which is the ratio of the molar concentration in cyclohexane to the concentration in water. The 3MI data have been corrected for the interaction between 3MI and water in cyclohexane (see text), and therefore K_v represents the ratio of the molar concentration of unhydrated 3MI in cyclohexane to the total molar concentration in water. Abbreviations: VF, volume-fraction free energy; MF, mole-fraction free energy; FH, Flory–Huggins-corrected volume-fraction free energy. Uncertainties in ΔG due to experimental uncertainties are approximately ±0.02–0.03 kcal/mol. Uncertainties in the partial molar volumes of 3MI and NMI of ±5% give rise to uncertainties of ±0.03–0.04 kcal/mol in volume-fraction free energies and to uncertainties of ±0.15–0.20 kcal/mol in the Flory–Huggins-based free energies (see text).

0.5 waters partition into cyclohexane with each 3MI, but it is possible that there is a distribution of hydrated states, some of which have more that one water. In such a case, the water-to-cyclohexane partition coefficient would be underestimated but the likely errors are probably not significantly larger than the experimental errors discussed in the text. Our calculated concentration of unhydrated 3MI in cyclohexane constitutes a lower limit. The maximum possible error would arise if only a small fraction of 3MI molecules were each hydrated by a large number of waters. In this unlikely case, the partition coefficient would be underestimated by a factor of 2, and the free energy of 3MI partitioning by 0.4 kcal/mol.

Radzicka and Wolfendon (1988) reported that no water copartitioned into cyclohexane with 3MI on the basis of NMR measurements in a series of nearly identical experiments using 3-methylindole, water, and cyclohexane. Radzicka and Wolfendon "extracted" the probe molecule from cyclohexane with water using vigorous mixing. In our experiments, we use a long equilibration with gentle mixing to ensure equilibrium. The concentration of water in the organic phase is measured directly using radioactive water. It is possible that in the experiments of Radzicka and Wolfendon (1988) complete equilibration of both the probe and water was not achieved or that their NMR technique was not sensitive enough to detect the increase in water content.

The uncorrected experimental data for NMI and 3MI partitioning between water and cyclohexane are shown in Figure 2. The results are listed in Table I, where the measured total concentration of 3MI in cyclohexane has been multiplied by the fraction of unhydrated 3MI. NMI does not interact with water in cyclohexane; therefore, no correction is required. The concentration of unhydrated 3MI in cyclohexane is about half of the total concentration (see Figure 1B). If the uncorrected 3MI concentration were used to calculate partitioning free energy, the value would be overestimated by 0.4 kcal/mol. If the fractional concentration of the unhydrated solute were smaller, the error caused by ignoring hydration in the solvent would be larger.

The free energies of 3MI and NMI transfer from water to cyclohexane calculated using the different partition coefficient units are listed in Table I and are shown in Figure 3. The values for 3MI include the cost of dehydrating the NH group in water and transferring the molecule into "dry" cyclohexane. This is the same cost as transferring hydrated 3MI into cyclohexane along with a water molecule. Pairs of curves calculated with the same units are parallel indicating that the entropy change of transfer ($d\Delta G/dT$) is the same for both

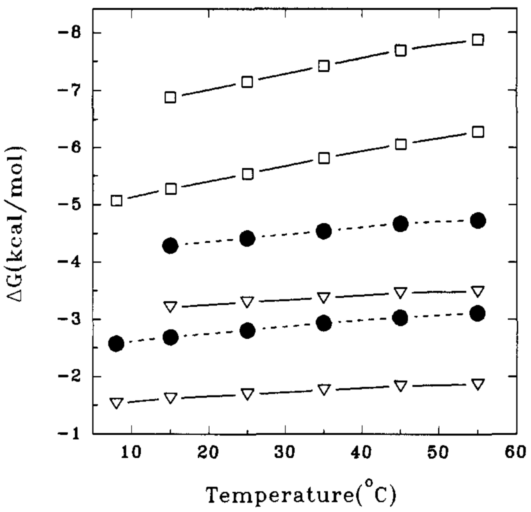

FIGURE 3: Free energies of partitioning of 3MI and NMI between water and cyclohexane. Free energies of 3MI and NMI partitioning were calculated as described in the text using volume-fraction (triangles), mole-fraction (circles), and Flory–Huggins-corrected volume-fraction partition coefficients (squares). The higher of each pair of curves calculated by the same method is for NMI partitioning. The difference between the curves is the same for each method. The uncertainties in free energies caused by experimental error are smaller than the symbols. Errors in the estimates of the partial molar volumes of 3MI and NMI could cause systematic errors in the Flory–Huggins-corrected volume-fraction free energies of ±0.15–0.20 kcal/mol (see text).

Table II: Thermodynamic Parameters for Water–Cyclohexane Partitioning of 3-Methylindole and *N*-Methylindole at 25 °C

method	ΔS (cal/(mol·K))	ΔH (kcal/mol)
	N-Methylindole	
VF[a]	8.00[b]	–0.91
MF	12.5	–0.69
FH	27.0	+0.88
	3-Methylindole	
VF	7.00	+0.40
MF	12.0	+0.77
FH	26.5	+2.36

[a] Abbreviations: VF, volume-fraction partition coefficients; MF, mole-fraction partition coefficients; FH, Flory–Huggins-corrected volume-fraction partition coefficients. These values were calculated from data between 15 and 35 °C only (see text). The uncertainty in ΔS due to experimental uncertainties is approximately ±1.4 cal/(mol·K) and the uncertainty in ΔH is approximately ±0.5 kcal/mol.

molecules. The values for ΔS for volume-fraction, mole-fraction, and Flory–Huggins-corrected volume-fraction partition coefficients are given in Table II.[2] The enthalpy changes listed in Table II were calculated from the temperature dependence of partition coefficients ($\Delta H = R(\mathrm{d}\ln K)/\mathrm{d}(1/T)$), where R is the gas constant and K is the partition coefficient. The enthalpy changes are all relatively small and contribute, at most, one-third of the total free energy change. These results are consistent with the hydrophobic nature of the water–cyclohexane partitioning of these two hydrophobic molecules (Privalov & Gill, 1988; Dill, 1990).

The entropy changes of partitioning, calculated with the same units, are the same for 3MI and NMI. Therefore, the differences in ΔG between 3MI and NMI partitioning are due to differences in ΔH of transfer. For all three pairs, the difference is constant with temperature and is equal to 1.61 ± 0.02 kcal/mol. This difference in enthalpy is due to the cost of removing the -NH- group from water, where it can form hydrogen bonds, and transferring it to cyclohexane in the absence of hydrogen bonds. These results indicate that the energetic cost of removing the indole -NH- group of tryptophan from water and immersing it in a nonpolar environment is lower than previously estimated. On the basis of solubility and partitioning data for a wide range of compounds, Engelman and Steitz (1981) estimated the cost for the burial of the tryptophan -NH- group as 3.0 kcal/mol [reviewed in Engelman et al. (1986)]. Jacobs and White (1989) obtained a value of 4.1 kcal/mol from the data of Roseman (1988) and Engleman and Steitz (1981). Here we *measure* the cost of moving the indole -NH- from water into cyclohexane and find that this value is only +1.6 kcal/mol. This means that the tryptophan -NH- group is considerably less polar than previously assumed.

A hydrogen bond with an energy of 1.6 kcal/mol is a weak interaction. Hermans et al. (1984) calculated the strengths of water–water, peptide–water, or peptide–peptide hydrogen bonds and found that they range from 5 to 7 kcal/mol. Spencer et al. (1978) measured the enthalpies of hydrogen bonding for a series of aromatic ring compounds containing N, NH, and C=O groups in solutions of chloroform and cyclohexane. These values range from 1.6 to 4.8 kcal/mol with an average value of approximately 3.5 kcal/mol for NH···C=O hydrogen bonds. Thus, it appears that the hydrogen bond between the indole NH group and water is an unusually weak one. As a result, the hydrogen bonding status of tryptophan in hydrophobic processes such as protein folding or insertion into lipid bilayers may be relatively small, especially if the Flory–Huggins-derived free energies are most appropriate. Even with an unsatisfied hydrogen bond, the contribution of tryptophan to hydrophobic processes is 5.5 kcal/mol (see Table I), which is equivalent to the contribution of phenylalanine (Sharp et al., 1991). If the hydrogen bond is satisfied, the contribution of tryptophan is 7.2 kcal/mol.

REFERENCES

Baldwin, R. L. (1986) *Proc. Natl. Acad. Sci. 83*, 8069–8072.
Ben-Naim, A., & Wilf, J. (1979) *J. Chem. Phys. 55*, 771–777.
Bevington, P. R. (1969) in *Data Reduction and Error Analysis for the Physical Sciences*, p 113, McGraw-Hill, New York.
De Young, L. R., & Dill, K. A. (1990) *J. Phys. Chem. 94*, 801–809.
Dill, K. A. (1990) *Biochemistry 29*, 7133–7155.
Engelman, D. M., & Steitz, T. (1981) *Cell 23*, 411.
Engelman, D. M., Steitz, T. A., & Goldman, A. (1986) *Annu. Rev. Biophys. Biophys. Chem. 15*, 321–353.
Erickson, H. P. (1978) *J. Mol. Biol. 206*, 465–474.
Fauchere, J. L., & Pliska, V. (1983) *Eur. J. Med. Chem. 18*, 369–375.
Flory, P. J. (1941) *J. Chem. Phys. 9*, 660–671.
Hermans, J., Berendsen, H. J. C., van Gunsteren, W. F., & Postma, J. P. M. (1984) *Biopolymers 23*, 1513–1519.
Huggins, M. L. (1941) *J. Chem. Phys. 9*, 440–449.
Jacobs, R. E., & White, S. H. (1989) *Biochemistry 28*, 3421–3437.
Janin, J., & Chothia, C. (1978) *Biochemistry 17*, 2943–2948.
Makhatadze, G. I., & Privalov, P. L. (1988) *J. Chem. Thermodyn. 20*, 405–412.

---

[2] For compounds like 3MI and NMI, there will be a heat capacity change (ΔC_p) with water–cyclohexane transfer (Privalov & Gill, 1988). Therefore, entropy and enthalpy changes will be dependent on temperature. Nonlinear least-squares curve-fitting was used to obtain ΔC_p. Using Flory–Huggins-based free energies, ΔC_p is –79 ± 15 for NMI and –47 ± 10 for 3MI. This range is similar to the values for benzene (–66 cal/(mol·K)) and cyclohexane (–97 cal/(mol·K)) (Privalov & Gill, 1988). In order to calculate ΔS and ΔH at 25 °C, we have used only the data between 15 and 35 °C, where the curvature is negligible relative to experimental uncertainties.

Murphy, K. P., Privalov, P. L., & Gill, S. J. (1990) *Science 247*, 559–561.

Nozaki, Y., & Tanford, C. (1971) *J. Biol. Chem. 246*, 2211–2217.

Privalov, P. R., & Gill, S. J. (1988) *Adv. Protein Res. 39*, 191–234.

Przybytec, J. T. (1980) in *Burdick and Jackson Solvent Guide*, Burdick and Jackson Laboratories, Muskegon, MI.

Radzicka, A., & Wolfendon, R. (1988) *Biochemistry 27*, 1664–1670.

Roseman, M. A. (1988) *J. Mol. Biol. 201*, 621–623.

Sharp, K. A., Nicholls, A., Friedman, R., & Honig, B. (1991) *Biochemistry 30*, 9686–9697.

Simon, S. A., McDaniel, R. V., & McIntosh, T. J. (1982) *J. Phys. Chem. 86*, 1449–1456.

Spencer, J. H., Gleim, J. E., Hackman, M. L., Blevins, C. H., & Garett, R. C. (1978) *J. Phys. Chem. 82*, 563–566.

Tanford, C. (1980) *The Hydrophobic Effect*, 2nd ed., Wiley, New York.

TRC Thermodynamic Tables. (1990) Thermodynamics Research Center, College Station, TX.

Weast, R. C. (1981) *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL.

Registry No. NMI, 603-76-9; 3MI, 83-34-1; Trp, 73-22-3.