Empirical Correlation Between Hydrophobic Free Energy and Aqueous Cavity Surface Area

(hydrophobic effect/hydrocarbons/water structure)

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ABSTRACT   The unitary free energy of transfer of a hydrocarbon molecule from a hydrocarbon solvent to an aqueous medium is a measure of the hydrophobic interaction in the aqueous medium. We have reexamined available data on this phenomenon and have confirmed that the free energy for saturated hydrocarbons is proportional to the surface area of the cavity created by the solute in the aqueous solution, with the same proportionality constant for linear, branched, and cyclic hydrocarbon molecules. The numerical value of the proportionality constant is uncertain because absolute and self-consistent area measurements are not available. We estimate that it falls between 20 and 25 cal/mole per Å² at 25° (for areas measured at the distance of closest approach of water molecules), which is significantly less than the figure of 33 cal/mole per Å² that has been assigned to the same parameter by Hermann [*J. Phys. Chem.* **76**, 2754–2759 (1972)]. A small discrepancy exists when similar data for homologous series of alkyl derivatives are compared with results based on hydrocarbons themselves, and possible reasons for it are discussed.

Hydrophobic free energy may be defined in terms of the unitary free energy of transfer of a non-polar solute from a non-polar reference solvent such as a liquid hydrocarbon to an aqueous medium (1). It can be determined from the equilibrium distribution between the two solvents. If X_{HC} and X_W are the equilibrium concentrations in mole fraction units in hydrocarbon and water, respectively, and μ^0_{HC} and μ^0_W are the corresponding standard chemical potentials,

$$\mu^0_W - \mu^0_{HC} = RT \ln X_{HC}/X_W \qquad [1]$$

represents the hydrophobic free energy. For pure hydrocarbons one may use the solubility of the hydrocarbon in water (X_s in mole fraction units). With $X_{HC} = 1$ Eq. 1 becomes

$$\mu^0_W - \mu^0_{HC} = - RT \ln X_s \qquad [2]$$

The necessity of using unitary units in these equations has been spelled out in several places (1–3). Use of these units eliminates cratic contributions to μ^0 so that $\mu^0_W - \mu^0_{HC}$ reflects only the internal free energy of the solute molecule and the free energy of its contacts with surrounding solvent molecules.

It is well established that the free energy of transfer defined by Eq. 1 is a linear function of the hydrocarbon chain length or the molar volume for normal saturated aliphatic hydrocarbons (1, 4). The same is true for homologous series of amphiphilic molecules with saturated n-alkyl chains (1, 5): for such molecules $\mu^0_W - \mu^0_{HC}$ represents the sum of the favorable free energy of interaction of water with the hydrophilic head groups (independent of alkyl chain length in a homologous

series) and the hydrophobic free energy of the alkyl chain. For branched or cyclic hydrocarbons, however, the free energy of transfer is less than for an unbranched chain with the same number of carbon atoms or the same molar volume. This is a reasonable result because the hydrophobic free energy arises from contacts between hydrocarbon and water at the solute-solvent interface and would thus be expected to be a function of the surface area of the hydrocarbon molecule (or alkyl chain in the case of an amphiphile) rather than chain length or molar volume.

This expectation has been tested in recent papers by Hermann (6) and by Harris et al. (7). Both papers reported that a good empirical correlation between free energy and surface area can indeed be made, incorporating cyclic, branched and unbranched chains in a single relation. In the procedure of Harris et al. (7) only relative surface areas were measured, so that an absolute value for the hydrophobic free energy per unit area could not be determined. Hermann (6), however, reported absolute area measurements and concluded that the hydrophobic free energy per unit area, at the distance of closest approach of water molecules to the surface of the cavity created by the solute, amounts to 33 cal/mol per Å² at 25° for saturated aliphatic hydrocarbons and to 30 cal/mole per Å² for aromatic hydrocarbons.

We have found it necessary to reexamine this question because the values of $\mu^0_W - \mu^0_{HC}$ determined from Hermann's surface areas and from the free energies per unit area given by him do not in fact agree with experimental values of $\mu^0_W - \mu^0_{HC}$. The analysis presented in this paper will confirm that proportionality between hydrophobic free energy and cavity surface area can be established. The free energy per unit area, however, turns out to be smaller than reported by Hermann: approximately 20 to 25 cal/mole per Å² for saturated aliphatic hydrocarbons and somewhat less than this for aromatic hydrocarbons. The uncertainty in these figures results from uncertainty in knowledge of absolute surface areas.

PROCEDURE

The experimental data used for the analysis are the solubilities of hydrocarbons in water measured by McAuliffe (4). The same data were used by Hermann (6). Computed cavity surface areas were taken directly from Hermann's tables. For hydrocarbons that can exist in several conformations, we have used only the most favored conformation. Surface areas for n-hexane, n-heptane, and n-octane are not provided by Hermann. His areas for ethane, n-propone, n-butane and n-
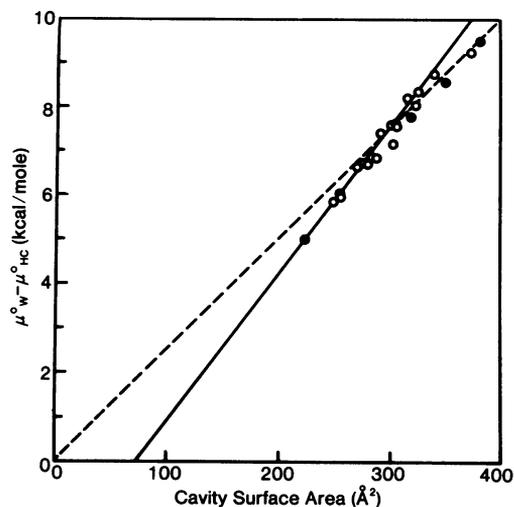
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FIG. 1. Unitary free energies of transfer of hydrocarbons from the pure liquid to aqueous solution at 25° plotted as a function of cavity surface area as determined by Hermann (6). The *solid line* is Hermann's line with a slope of 33 cal/mole per Å². The *broken line* passing through the origin has a slope of 25 cal/mole per Å². The ●s represent data point that were not included in Hermann's analysis.

pentane in the most favored, fully-extended conformation differ by identical increments of 31.8 Å² per CH_2 group, and the areas for the three higher hydrocarbons were obtained by using this figure.

A simple method for determining cavity surface areas by packing spheres representing water molecules around molecular models has been described by Harris *et al.* (7). We have used the method as described and have obtained excellent agreement with their results. This method provides areas of alkyl groups rather than whole hydrocarbon molecules, and does so in relative units, the *tert*-butyl group being unity. We have used the method for the determination of the areas
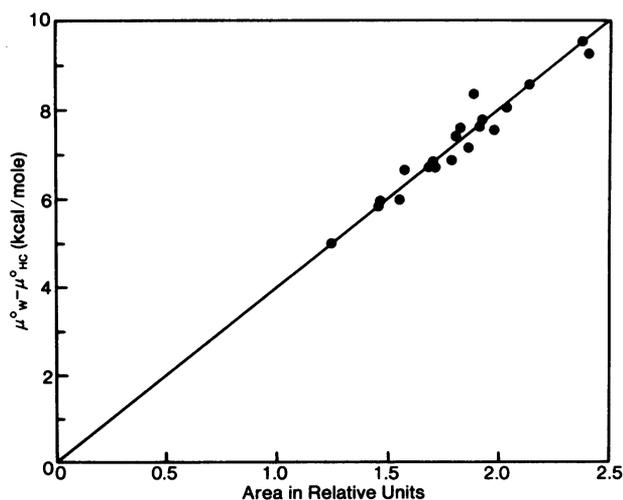

FIG. 2. The same data as in Fig. 1, plotted as a function of relative area, determined by the method of Harris *et al.* (7). Most of the areas were determined by adding the appropriate value for the addition of a proton (see *text*) to the relative area of the corresponding alkyl group as tabulated by Harris *et al.* (For example, the *tert*-butyl radical is used to define unit area on the relative scale. The corresponding hydrocarbon molecule, isobutane, has a relative area of 1.45.)

for whole molecules by extending the packing procedure to measure the additional area created by adding a hydrogen atom to the end of the alkyl group. Since the method as described by Harris *et al.* (7) involves placing the cross-section of the terminal atom of the alkyl chain on a flat surface, the additional area should be the same for all alkyl radicals that terminate in a primary C atom (i.e., a CH_2 group). However, somewhat larger values would be expected if the terminal atom is a branching point. We have made measurements for several molecules and obtain an increment in area of 0.28 relative units for addition of a hydrogen atom to a terminal CH_2 group. This is a reasonable result when compared to the area of 0.51 relative units for a CH_3 group. The same result can be obtained directly from the data of Harris *et al.* by using appropriate additivity relations, e.g., the sum of the areas of two CH_3 groups must be the same as the area obtained by adding a hydrogen atom to a C_2H_5 group. For adding a hydrogen atom to a alkyl chain terminating in a secondary or tertiary C atom we obtain area increments of 0.35 and 0.45 relative units, respectively.

RESULTS

We have used all of McAuliffe's experimental data (4) for solubility of liquid saturated aliphatic hydrocarbons. We have included propane, for which McAuliffe gives only a solubility referred to the gaseous state. It is readily converted to the liquid solubility by use of thermodynamic data for vaporization, with a resulting uncertainty no greater than that of the experimental data themselves. We have included McAuliffe's data for *n*-hexane, *n*-heptane, and *n*-octane, which were omitted in Hermann's analysis (6).

In Hermann's analysis the solubility (*s*) was expressed in molar units. An equation of the form

$$RT \ln s = b_0 A + c_0 \qquad [3]$$

was employed where A is the cavity surface area, and b_0 and c_0 are constants. The hydrophobic free energy per unit area was equated with b_0, but the constant c_0 was not evaluated. Thus it could not be shown that the assigned value of b_0 leads to a reasonable limit of the free energy at zero cavity area. We have replotted the data in Fig. 1 with solubility expressed in mole fraction units, which simply involves a shift in the ordinate scale. In these units the ordinate is by Eq. 2 equal to $\mu^0{}_W - \mu^0{}_{HC}$ in unitary units. If the hydrophobic free energy is strictly proportional to A the plot should not only be linear but must pass through the origin. It is evident that Hermann's line with slope 33 cal/mole per Å² does not do so. If this line were accepted it would indicate that non-polar solutes of the size of He or Ne (cavity surface area somewhat below 100 Å²) would exhibit no hydrophobic effect, which is contrary to fact.

The results also show that Hermann's line is prejudiced by the omission of the results for *n*-hexane, *n*-heptane, and *n*-octane. Even if the problem of the intercept at A = 0 is ignored, significant deviation from linearity is introduced into the plot when these data are included.

We have included only the most favored conformations for hydrocarbons with multiple conformational states, but have made similar plots using the weighted average conformations (6) and the results are not significantly altered. The conclusion from Fig. 1 is either that the computer program used to obtain the values of A does not correctly represent the solute-solvent contact area or else that this area is in fact not the variable that determines hydrophobic free energy.

To obtain further evidence on this question we have re-plotted the same results in Fig. 2 using relative areas (A') calculated by the method of Harris *et al.* (7). This method depends on manual packing of model water molecules around a molecular model and is incapable of the precision of a computer method, but would appear to contain no possible source of systematic error. It is evident from the figure that $\mu^0_W - \mu^0_{HC}$ is in fact linearly related to A' and that the straight line through the points passes through the origin without straining the data. The single deviant point is for 2,4-dimethylpentane. Points for other branched hydrocarbons (2,2-dimethylbutane, 2,2,5-trimethylhexane) and for cyclic hydrocarbons show no deviations. The result suggests that the non-adherence of the data of Fig. 1 to the expected linear relation might well result from inappropriate values for the cavity areas.

The slope of the line of Fig. 2 is close to 4000 cal/mole per relative area unit. To obtain an absolute value for the dependence of $\mu^0_W - \mu^0_{HC}$ on surface area requires that at least one absolute value of A can be established. Since the surface area of methane should be the easiest to measure without ambiguity, we have assumed that Hermann's area for methane (152.4 Å^2) is correct. An approximate calculation can be made by assuming that methane can be represented as a sphere with radius equal to the van der Waals radius of a methyl group (2.0 Å). This calculation leads to an estimated area at the distance of closest approach of water molecules of 154 Å^2. A similar approximate estimate for neopentane (319 Å^2) is consistent with the relative areas obtained by the method of Harris *et al.*, but suggests that Hermann's area for this hydrocarbon is too small.

Assuming 152.4 Å^2 for the area of CH$_4$ leads to a conversion factor of 192 Å^2 per relative area unit and to a proportionality of 21 cal/mole per Å^2 between hydrophobic free energy and cavity surface area. However, even if other computer-calculated areas were assumed to be correct, the maximal value for the proportionality constant that could have been obtained is 25 cal/mole per Å^2, as is evident from the *broken line* of Fig. 1.

Area calculations by the method of Harris *et al.* have not been made for aromatic hydrocarbons. When the results of Hermann are replotted in the manner of Fig. 1 the maximal slope of a line through the origin is about 20 cal/mole per Å^2.

DISCUSSION

We conclude that the hydrophobic repulsive free energy between water and hydrocarbon chains is proportional to the area of contact measured at the distance of closest approach of water molecules. The proportionality constant is 21–25 cal/mole per Å^2 for saturated aliphatic hydrocarbons and ≤ 20 cal/mole per Å^2 for aromatic hydrocarbons. The uncertainty in these figures is chiefly the result of uncertainty in the measurement of contact area. The result is applicable to cyclic and branched hydrocarbons as well as to the series of n-alkanes.

Our result is of course purely empirical, and is intended only for the purposes of making estimates of the expected hydrophobicity for complex molecules. Theoretical models for the hydrophobic effect are not yet sufficiently well developed to decide whether strict proportionality between free energy and cavity surface area is to be expected on a theoretical basis. One trivial reason for anticipating some deviation from the line drawn in Fig. 2 or from the broken line drawn in Fig. 1 is

that extrapolation of any method of area measurement (at the distance of closest approach of solvent molecules) does not lead to zero area for a solute of zero volume. This particular factor is within the experimental error of the data, but there may be other theoretical reasons for non-linearity or for a finite value for $\mu^0_W - \mu^0_{HC}$ at zero area (R. B. Hermann, personal communication).

It is worthwhile to draw attention to the fact that the dependence of $\mu^0_W - \mu^0_{HC}$ on the size of the alkyl chain obtained from the hydrocarbon solubility data used in this paper is about 7% higher than similar data based on the distribution of alkyl derivatives between water and hydrocarbon or other non-polar organic solvent. This discrepancy does not depend on a knowledge of absolute areas. For example, the slope of Fig. 2 is 4000 cal/mole per area unit, whereas Harris *et al.* (7) obtained a slope of 3700 cal/mole per area unit for the transfer of dextromorphan alkyl sulfates (straight, branched and cyclic alkyl groups) from water to 25% CHCl$_3$ in CCl$_4$. When the solubility data for n-alkanes are plotted as free energy versus alkyl chain length (1) one obtains a slope of 884 cal/mole per added CH$_2$ group. Similar data for the transfer of n-alkyl carboxylic acids from water to heptane yield a slope of 825 cal/mole per added CH$_2$ group (3), and we (J. A. Reynolds, D. G. Gilbert, and C. Tanford) shall show in a subsequent paper that a similar result is obtained for aliphatic alcohols. The origin of this discrepancy has not been explained. It seems inherently unlikely that the effect of an increase in hydrocarbon–water contact area (e.g., in comparing myristyl with dodecyl derivatives) can be influenced by a polar group attached a long distance away. It is perhaps possible that the discrepancy arises from the different reference states used for the non-aqueous solvent. In the data for hydrocarbons the reference state is the pure liquid hydrocarbon. In the data for other alkyl derivatives the reference state is an infinitely dilute solution in a liquid hydrocarbon.

It might also be pointed out that present conceptualization of the origin of the hydrophobic effect does not suggest that enthalpies and entropies of transfer will necessarily be regular functions of contact area. The anomalously high heat capacity associated with the hydrophobic effect suggests that the water molecules surrounding a hydrocarbon chain or other non-polar cavity can exist in two or more states of nearly the same free energy but with different enthalpies and entropies (1). The *shape of the cavity* could well affect the distribution between these states, leading to unpredictable variations in enthalpy and entropy without significant perturbation of the linear dependence of free energy on cavity surface area.

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