On the Probability of Finding a Water Molecule in a Nonpolar Cavity

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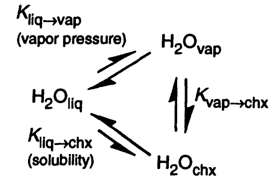
Measurements of solubility and vapor pressure indicate that the cost of making a cavity in a nonpolar solvent that is large enough to accommodate a single water molecule is just balanced by the attraction between the water molecule and the walls of the cavity. As a result of this unexpected coincidence, the equilibrium constant for entry of an isolated water molecule from the vapor phase into cyclohexane is almost exactly unity at room temperature. Molecules smaller than water prefer the vapor phase, whereas molecules larger than water prefer cyclohexane. In equilibrium with liquid water, the tendency of single water molecules to enter small nonpolar cavities, such as may be present in mutant proteins, is expected to be vanishingly small.

Interactions between biological molecules usually involve the stripping of solvent molecules from between the reacting partners; therefore, variations in the free energy of interaction with solvent water exert a major influence on the strength and specificity of inter- and intramolecular interactions (*1*). The converse process, in which single water molecules enter nonaqueous environments, is also of biological interest, in relation to the mechanism by which water is transported across membranes (*2*) and the analysis of forces involved in recognition between biological molecules.

Functional groups are sometimes deleted from a ligand or a protein in an effort to analyze their contributions to binding, catalysis, or protein stability. If a cavity resulting from deletion were large enough to accommodate water, then the effects of deletion would be influenced by any tendency of water to compete for a place in that cavity (*3, 4*). Similar considerations apply to changes in protein stability that arise when cavities are introduced by the mutation of internal amino acid residues. Electron density maps have revealed that in several mutant proteins, water appears to be absent from nonpolar cavities (*5–8*). It would be desirable to have quantitative information concerning the tendencies of such cavities to attract, or exclude, water molecules under physiological conditions.

Are water molecules attracted or repelled by nonpolar surroundings? An attractive force has been shown to exist across the interface between liquid octane and liquid water (*9*) and between isolated methane and water molecules in the vapor phase (*10*). It has not been clear, however, whether this attraction is strong enough to permit an isolated water molecule to overcome the self-cohesive properties of the nonpolar solvent, as is necessary if it is to enter solution. An approximate answer to that question can be obtained by comparing the solubility of water in a nonpolar solvent such as cyclohexane, in which water exists as a monomer at the low concentrations at which it is present at saturation (*11*), with its "solubility" in the vapor phase, in which water exists as a monomer at atmospheric pressure (*12*). The scheme below shows that the ratio of these solubilities, expressed as moles per liter in each phase, is equivalent to the equilibrium constant $K_{vap \rightarrow chx}$, expressed in terms of the number of molecules per unit volume in each phase, for transfer of single water molecules from the vapor phase to cyclohexane.



We determined the solubility of water by stirring cyclohexane (10 ml) with ^3H_2O (0.05 ml, 0.1 mCi) in a 20-ml scintillation vial immersed in a water bath for various intervals and then removing aliquots of the upper phase after the phases had been allowed to separate. With magnetic stirring at moderate speed, the half-time for approach to equilibrium was about 4 min. Solubilities were routinely determined after stirring for 40 min, when equilibrium had been established. Vapor pressures of water were obtained from standard tables (*13*).

The concentration of monomeric water in the vapor phase closely approaches its concentration in saturated cyclohexane (Fig. 1). This indicates that the equilibrium constant for the transferral of a water molecule from the vapor phase to cyclohexane approaches unity (*14*).

Figure 2 shows the solubility of water in cyclohexane and the concentration of water in the vapor phase, plotted as a logarithmic function of the reciprocal of the absolute temperature. At 26°C, where these lines intersect, the equilibrium constant for transfer from the vapor phase to cyclohexane becomes unity. The similarity of the two van't Hoff plots indicates that transfer is accompanied by only minor changes in enthalpy (–1.4 kcal/mol) and entropy ($T\Delta S$ = −1.4 kcal/mol).

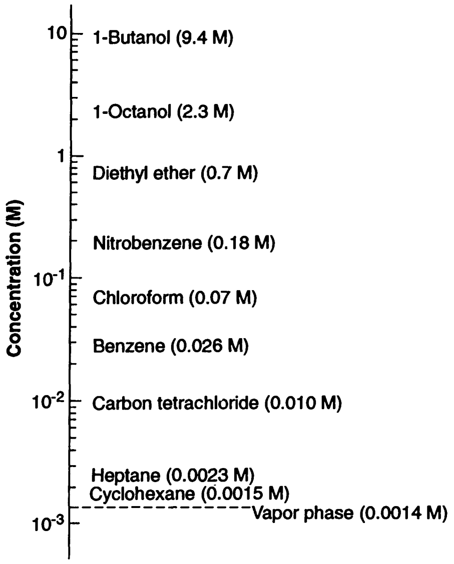


Fig. 1. Concentration of water in various solvents and in the vapor phase, in equilibrium with liquid water at 25°C.

At 26°C, the cost in free energy of making a cavity in a liquid hydrocarbon large enough to accommodate a water molecule just matches a water molecule's free energy of attraction to the walls of the cavity. This equivalence seems remarkable in view of the widely varying behavior of other solutes

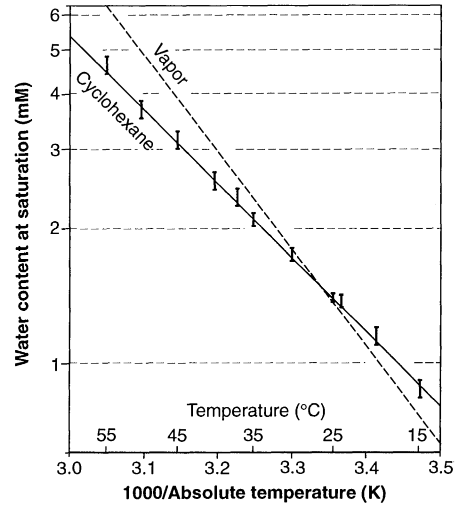


Fig. 2. Concentration of water in cyclohexane and in the vapor phase, in equilibrium with liquid water, plotted as a function of the reciprocal of the absolute temperature.

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Table 1. Equilibria of transfer from vapor to cyclohexane at 25°C. The values are from the solubilities collected by Wilhelm and Battino (*20*), except that for water, which is from the present experiments, and that for cyclohexane, which is calculated from the vapor pressure of cyclohexane at 25°C.

Solute	$\Delta G_{\text{vap}\rightarrow\text{chx}}$ (kcal/mol)
Helium	2.17
Neon	1.94
Argon	0.69
Methane	0.23
Krypton	0.02
Water	−0.04
Xenon	−0.82
Ethane	−0.94
Propane	−2.05
Butane	−2.77
Cyclohexane	−4.35

(Table 1). Molecules smaller than water, such as helium and hydrogen, strongly prefer the vapor phase. Larger molecules, such as xenon or propane, much prefer the hydrocarbon phase. Krypton, with a molecular surface area similar to that of water (*15*), also shows a distribution coefficient of approximately unity for transfer from the vapor phase to cyclohexane (*16*).

Is water, in equilibrium with liquid water, expected to occupy a nonpolar cavity created in the interior of a protein by mutation? An approximate, but decisive, answer to this question can be obtained for any nonpolar region in the interior of a protein molecule whose properties are assumed to resemble those of a liquid hydrocarbon (*17*). In equilibrium with liquid water, cyclohexane contains 1.5×10^{-3} M water. At that concentration, one water molecule is present in a volume of about 1.1×10^{6} Å^3 of solvent. Accordingly, the probability of finding a water molecule in any particular volume of solvent just large enough to accommodate a water molecule would be 1 in 60,000 (*18*). If a cavity had been created in the solvent before water entered (in an operation comparable with the creation of a cavity in a protein by mutation), then that chance would increase. The cost in free energy of opening a cavity of this size in a protein molecule is roughly 0.7 kcal (*19*). After correction for this effect, the chance of finding a water molecule that is in equilibrium with liquid water, in a preformed nonpolar protein cavity just large enough to accommodate water, is of the order of 1 in 20,000. That probability would increase markedly, of course, if the water molecule were able to make contact with a polar group or with another water molecule.

These results lead to the inference that a single water molecule is highly unlikely to be found in a small nonpolar cavity in a protein. Individual water molecules pass between the vapor phase and a nonpolar condensed phase, however, with remarkable equanimity.

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14. Other experiments show that single water molecules behave similarly toward aliphatic hydrocarbon solvents ranging from C_5 to C_{17} and toward perfluorocarbon solvents ranging from C_6 to C_{10}, with equilibrium constants ranging from 0.7 to 1.6 for transfer from the dilute vapor phase to the organic solvent (R. Wolfenden and A. Radzicka, unpublished results).
15. The molecular surface area of both molecules, as estimated by the method of J. L. Pascual-Ahuir and E. Silla [*J. Comput. Chem.* **6**, 1047 (1990)] is about 35 Å^2 (D. Doyle, unpublished results).
16. The comparable behavior of water and krypton may appear surprising. However, earlier work has shown that vapor-to-cyclohexane distribution coefficients of other molecules are remarkably insensitive to differences in solute polarity [A. Radzicka and R. Wolfenden, *Biochemistry* **27**, 1664 (1988)].
17. The possibility that such a resemblance exists, at least to a limited extent, is supported by the observation that differences in side-chain free energies of transfer from cyclohexane to water are numerically similar to changes in free energy of denaturation that result from mutating nonpolar side chains in protein interiors [G. D. Rose and R. Wolfenden, *Annu. Rev. Biophys. Biomol. Struct.* **22**, 381 (1993)].
18. This probability was estimated by dividing the approximate volume of a water molecule, 20 Å^3 [taken as the volume of a sphere with a surface area of 35 Å^2 (*15*)], into 1.1×10^{6} Å^3, the volume of distribution of each water molecule in water-saturated cyclohexane.
19. The cost in free energy of opening a nonpolar cavity in the interior of a protein molecule is probably in the neighborhood of 20 cal/Å^2 (*5*), equivalent to an equilibrium constant of 0.3 for opening a cavity with an area of 35 Å^2 (*15*). If the cost in free energy of opening such a cavity had been paid in advance, then water's tendency to enter the cavity would be enhanced by a factor of about 3.
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Surface Dating of Dynamic Landforms: Young Boulders on Aging Moraines

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The dating of landforms is crucial to understanding the evolution, history, and stability of landscapes. Cosmogenic isotope analysis has recently been used to determine quantitative exposure ages for previously undatable landform surfaces. A pioneering application of this technique to date moraines illustrated its considerable potential but suggested a chronology partially inconsistent with existing geological data. Consideration of the dynamic nature of landforms and of the ever-present processes of erosion, deposition, and weathering leads to a resolution of this inconsistency and, more generally, offers guidance for realistic interpretation of exposure ages.

The surface of the globe is continually changing because of the interplay between degradational processes that tend to lower the landscape and constructional processes that tend to elevate it. Understanding these processes and the rates at which they operate is of value to both the scientific community and society, because the form and stability of land surfaces impact and are impacted by many human activities. Considerable information about rates of surficial processes can be extracted from ages of landform surfaces. Until recently, these ages have been approximated by dating material above or beneath the surface or by estimating the time required to account for the observed degree of weathering and erosional modification of the surface. In general, it has not been possible to date landform surfaces directly and quantitatively. In this context, the development of exposure-age dating of rock surfaces based on the progressive accumulation of diverse isotopes produced by collisions with cosmic rays (*1*, *2*) has been welcomed by geomorphologists. In this report, we focus attention on dating moraines because they have been the subject of several cosmo-

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