A New Method of Solvation Analysis: Applications to Quinones

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A new method of analysis of the factor contributing to solvation of small molecules is described. Approximations of the energetic contributions to the entry of a molecule into water have been derived from partition coefficient data of solutes and selected derivatives from a multiplicity of solvents. These include taking separate account energy of the cost of introduction of a molecular cavity in water, the strength of solute-water dipolar interactions, and the strengths of hydrogen bond formation with water of the lone pairs and hydroxylic hydrogens associated with the molecule. In this report the solvation-free energies of benzoquinone and hydroquinone in water are described. We also consider the solvation of the semiquinone anion and show it to be fundamentally different from that of either the quinone or hydroquinone; this is discussed as a potentiality for selective binding ("solvation") of quinone, semiquinone and hydroquinone in sites of redox catalysis such as those found in the photosynthetic reaction center.

Introduction

Over the past few years extensive studies have been made in many laboratories to determine what are the factors that govern the binding of quinone cofactors and their antagonists to catalytic sites of bacteria, mitochondria and chloroplast systems. However, it has become increasingly clear that a more highly resolved description of the solvation of these molecules in water and other solvents is needed if an energetic description of the cofactor/antagonist interaction with the site ("solvation") at an atomic level is to be achieved. To this end, like other investigators before us [1–13], we have explored the partition properties (transfer-free energies) between organic solvents and water of a wide variety of related solutes with the intent to resolve the features important to their interactions with different environments. This paper describes a new approach to quantify the interaction of solutes with several solvent environments with a view to a better understanding of their interactions with water, membrane and protein milieux. Strategies have been devised to dissect the solvation of these molecules into three energetic divisions: 1) solute cavity formation in water, 2) solute-solvent electrostatic (dipolar) interactions and 3) hydrogen bonds between water and solute lone pairs and water and hydrogens.

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Rationale

The cavity

Langmuir, in 1917, demonstrated that the tendency of amphophilic molecules (fatty acids) to partition themselves between an air-water interface was directly related to the number of methylene groups they contain [6, 7]. The contribution made by water's unique reluctance to accommodate molecules has given rise to the term "hydrophobicity" [7, 8]. Compared to other solvents such as alkanes, alkanols, chloroform or ethers, water exacts an extra energetic cost for the introduction of a solute into its bulk phase [9]. The source of this effect lies in the highly associated structure of liquid water which upon introduction of a solute is disturbed, leading to unfavorable ordering (decrease in entropy) of water molecules around the solute [8]. For a simple hydrocarbon solute entering water, this entropic term, contributing unfavorable (positive) free energy, dominates over any enthalpic interactions affording favorable (negative) free energy to the transfer process. Indeed, the positive value of the cavity-free energy term is significant, and, for many molecules of biological interest such as native cofactors, inhibitors or herbicides, is of comparable or larger magnitude than the summed negative-free energy interactions arising from elec-

trostatic or hydrogen bond contributions. The resultant transfer-free energy of a molecule from some apolar phase into water, the basis of the experimentally measured partition coefficient, is the difference of two large opposing effects. Unfortunately, the large majority of work done to understand the forces governing the transfer of solutes into and out of water and into biological milieux has relied on such partitioning data [3–5, 9, 10]. Thus, the first step in our analysis is to obtain a practical evaluation of solute cavities so that a more accurate characterization of the other forces contributing to partitioning behaviour of a solute can be achieved.

Early researchers attempted to address the cavity or hydrophobicity problem by utilizing solvents such as methanol and ethanol to approximate the dipolar and hydrogen bonding potential of water without is associated high degree of internal structure [3, 5, 10]. However, this approach is not without practical and theoretical difficulties. For instance, partition coefficients from short chain alcohols cannot be obtained practically since these solvents are miscible with water; in these cases solute solubilities in the two phases have been used as an alternative [3, 5, 10]. For longer chain alcohols such as butanol, pentanol and hexanol, partition coefficient measurements can be performed, but the high level of mutual solubility of these alcohols and water tends to undermine the quantitative aspects of a two phase model. While water content in even longer chain alcohols remains significant, the last thirty years has been octanol become the solvent of choice. It appears to us that over time the reasoning behind the choice of octanol and the other alkanols has changed; now they are used as a representative first approximation of an average protein or membrane interior, or as a guideline to the partitioning of drugs and herbicides in cells and tissue [4, 9, 11–13]. For our purposes, however, any alcohol-water system is not suitable because alkanols have their own propensities (quantitatively not yet fully understood) to interact with solutes *via* dipolar and hydrogen bonding interactions, the presence of which compromise our wish to assess the interaction strengths of such solutes with water. Thus, the logical choice of organic phase for our purposes is the simple alkane.

Alkanes, being limited to dispersion interactions, and incapable of forming the specific solute-solvent complexes of the type found in water (or alkanols), provide a practical system from which the solvation properties of a single phase can be determined [9]. Our determination of the cavity contribution is based on the premise that the partitioning of non-interactive molecules, such as alkanes, between water and hexane is attributable solely to its effect on the structure of surrounding water molecular environment. Therefore, the cavity contribution of any solute (RXH_n; R is an organic residue, where X is a heteroatom containing lone pair(s) and H_n represents hydrogens associated with the heteroatomic center) should be attainable from the partition behaviour of a non-interactive structural equivalent ($RCH_{3-n}Me_n$). This kind of approach has also been explored by Fleischmann and Brooks [14] to determine the "interaction strengths" with water of the hydroxyl groups of ethanol and methanol; thus, for example, the cavity model chosen for ethanol is propane.

The contributions from dipoles

Molecular dipoles

We are currently exploring several avenues to apply data derived primarily from partitioning experiments to resolve separately the dipolar contributions to the free energy of solvation of a molecule in water. As a first step, we have speculated that chloroform, and to some extent ethers, interact with a wide variety of solutes predominantly through dipolar interactions. The protocol we have used to test this idea aims, in effect, to obtain a partition coefficient for a solute between hexane and chloroform. Since it is not feasible practically to obtain direct alkane to chloroform transfer-free energies, their values have been estimated through subtraction of water to chloroform and water to alkane transfer-free energies of the molecule in question. If the transfer-free energies so obtained reflect a reasonable quantitation of the dipolar interaction of a solute with its solvent environment, then the hexane/chloroform transfer-free energy of the solute should be proportional to its dipole moment within chloroform. The results we present here show that hexane/chloroform transfer-free energies are proportional to the published gas phase dipole moments of a series of similar, sized molecules, and hence demonstrate that with such a line

of calibration, the dipolar interaction strength of a solute in chloroform can be estimated from published molecular gas phase dipole moments. We are aware, however, that the dipolar interaction strength of a solute in chloroform will, in general, be less than that encountered in water, a solvent of much higher dielectric constant. Therefore, despite the value of the hexane/chloroform system in providing support for our idea, the resolved dipolar contribution to the transfer-free energy of a molecule into chloroform thus obtained is expected to be quantitatively an underestimate of that involved when the same molecule enters water. Hence, in this report, we also present preliminary results regarding the construction of a similar calibration line for solute/water dipolar interaction strengths.

Local dipoles

Although molecules such as *p*-benzoquinone and hydroquinone do not possess a permanent molecular dipole moment, the local dipolar characters of, respectively the carbonyl and hydroxyl groups are expected to contribute significantly to their overall transfer-free energies. Here we consider the possibility of obtaining approximate transfer-free energies contributed by these local dipoles by calculating bond moments from *ab initio*-derived atomic charge densities (G. Greco and Y. Martin, personal communication) and using the dipole moment/free energy calibration curve described above.

Hydrogen bond interactions

The methods described in the preceding sections permit evaluation of the cavity and dipolar contributions to the solvation of small molecules. The remaining important contributions will result from any hydrogen bond interactions existing between the solute and the solvent. Since hydrogen bonds arise separately from the lone pair (X) and hydrogens (H_n) of a substituent group (XH_n) present on the solute, we have devised a method to obtain a rough resolution of their individual contributions. This is achieved by comparing the alkane/water partition coefficient for RXH_n with that for the molecule resulting after removal of the H_n term by "methylation" of X, *i.e.* of RXH_n with $RXMe_n$. By taking steps to account for changes in cavity, dipole moment and lone pair basicity resulting from such "methylation", the difference in transfer-free energies between the molecules RXH_n and $RXMe_n$ yield the contributions due to the hydrogens and, by difference, the lone pairs.

An important source of modulation of the lone pair and hydrogen interaction strengths with solvent are their basicities. Since there is a large electrostatic contribution to the hydrogen bond, a correlation basicity of the solute lone pair on X with the free energy of hydrogen bond formation with water is expected. This has been shown to be the case by Arnett and Taft *et al.* who correlated the lone pair basicity of a series of potential hydrogen acceptors with their observed enthalpy of hydrogen bond formation with *p*-fluorophenol [15–17]. In this report we show, not only that the magnitude of the contribution to solvation from the hydrogen bond between water and the lone pairs of X is proportional to their pK, but also that the contribution from the hydrogen bond between water and the H_n moieties of XH_n is inversely proportional to the pK on the lone pairs of X.

Materials and Methods

The transfer-free energies (ΔGtr values) were calculated from log P values (P = partition coefficient) obtained from Hansch and Leo [12] and using the relationship $\Delta G\text{tr} = -R\text{T}\ln P$. In calculating the free energy of transfer, from the data in reference [12], a standard state of 298 K is assumed. Gas phase dipole moments were taken from ref. [19]. pK values were taken primarily from refs. [20–24]. Cavity contributions for quinone, semiquinone, and hydroquinone were estimated from surface area calculations of similar compounds utilizing the methodology of Lee and Richards and a value of 25 cal A^{-2} [25, 26].

Results and Discussion

Fig. 1 shows, for a number of compounds, an excellent correlation between the chloroform (diethyl ether)/hexane transfer-free energies and their gas phase dipole moments ($R^2 = 0.98$). This provides considerable evidence that the predominant contribution to the transfer process arises from the dipolar character of these molecules. An extension of this investigation to the dipolar forces in water is more limited due to the fact that most compounds form hydrogen bonds with water. How-

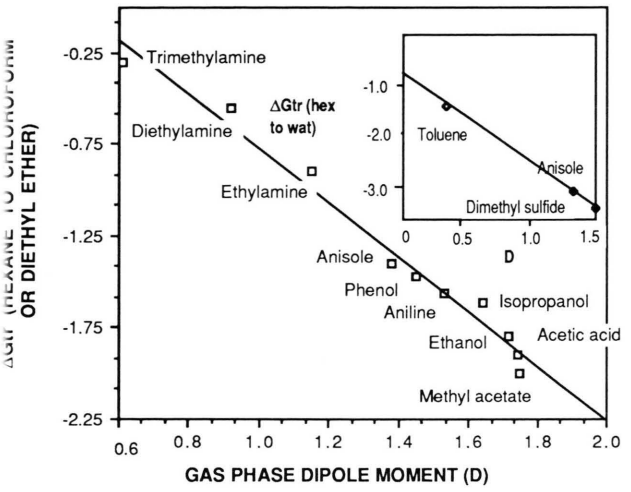

Fig. 1. Relationship of hexane to chloroform (or diethyl ether) transfer-free energies (ΔGtr) with gas phase dipole moments. Diethyl ether was used to obtain a measure of the dipolar forces for amines and pyridine, for which there is a possibility of hydrogen bonding or chemical reaction in chloroform. A slope of 1.41 kcal mol^{-1} D^{-1} was obtained by linear least squares regression analysis of the data. The insert to Fig. 1 shows preliminary evidence for the relationship of hexane-water transfer-free energies with gas phase dipole moment of compounds for which no hydrogen bond is expected.

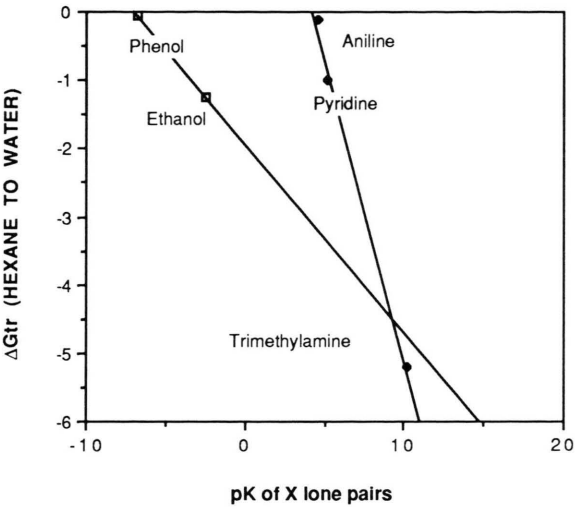

Fig. 2. Dependency of ΔGtr (hexane to water) on the pK of the X lone pairs. Separate correlations were observed for oxygen and nitrogen lone pairs interacting with water (for which slopes of 0.26_5 and 0.88 kcal mol^{-1} pK^{-1} were obtained respectively).

ever, the insert to Fig. 1 shows a preliminary indication that compounds which do not explicitly hydrogen bond with water (unpublished work) express such a relationship. As expected, the solvation as a function of gas phase dipole moment in water is quantitatively different from that in chloroform; for example, the interaction strength for anisole is -1.7 kcal mol^{-1} greater in water that it is in chloroform.

Fig. 2 correlates the pK of solute lone pair(s) (X) with its (their) derived interaction strengths with water for compounds containing either oxygen or nitrogen. Slopes of -0.265 and -0.88 kcal mol^{-1} per pK unit are obtained for, respectively, an oxygen or nitrogen lone pair interacting with water. Fig. 3 shows a similar relationship for the H_n moiety; the basicity of X correlates well with the strength of interaction of H_n with water independent of the nature of X. A slope of $+0.17$ kcal mol^{-1} per pK unit for oxygen and nitrogen was obtained for the interaction strength of H_n with water.

Table I demonstrates the application of our approach to *p*-benzoquinone and hydroquinone. The Table lists for the two molecules their cavity equivalent, dipolar contributions and hydrogen bond contributions from both X and the associated hydrogens. The dipolar contributions for these molecules were obtained from calculated carbonyl (1.22 D) and hydroxyl (1.35 D) bond moments,

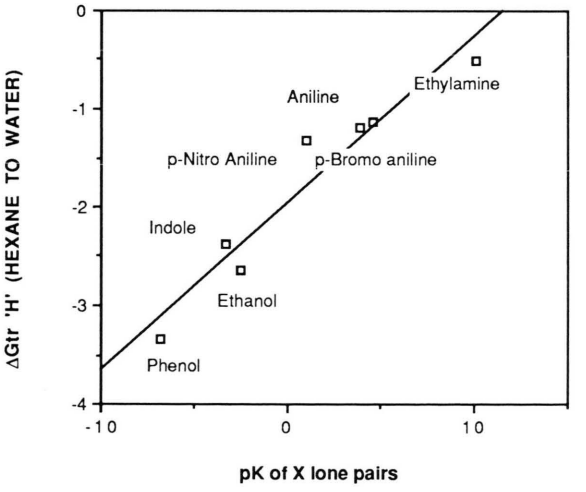

Fig. 3. Dependency of ΔGtr of the hydrogens of XH_n on the pK of X lone pairs. A slope 0.17 kcal mol^{-1} pK^{-1} was obtained from linear least squares analysis of the data set.

Table I. Benzoquinone and its parameters for solvation analysis.

Benzoquinone species	ΔGtr [kcal/mol]
p-Benzoquinone	
a. Cavity equivalent	4.93
b. Dipolar term ×2 (carbonyl bond moment)	−4.96
c. Lone pair H-bond term (2 carbonyl oxygen)	0.00
Summation of a, b, c	0.03
d. Measured partition coefficient	0.53
Hydroquinone (neutral)	
a. Cavity term	6.32
b. Dipolar term × 2 (hydroxyl bond moment)	−5.34
c. Lone pair H-bond term (2 phenolic oxygens)	0.00
d. Hydrogen H-bond term (2 phenolic hydrogens)	−7.36
Summation of a, b, c, d	−6.38
e. Measured partition coefficient	−5.73
Semiquinone (anion-radical)	
a. Cavity equivalent	+5.26?
b. Lone pair H-bond term (per lone pair)	−3.16?
c. Dipolar term ×2	?

All data refer to the movement of the solute molecule into water.

using the calibration curve shown in the insert to Fig. 1. The hydrogen bond contributions were determined from Fig. 2 and 3 using an oxygen lone pairs pK of −7.6 for benzoquinone and of −6.8 for hydroquinone. As can be seen from Table I, the local electrostatic contribution accounts for most of the solvation attributable to the lone pairs of the quinone and hydroquinone; consequently, hydrogen bonding by the lone pairs of *p*-benzoquinone and hydroquinone to water is revealed as being negligible. In contrast, hydrogen bonding to the H of the hydroquinone is substantial. It is important to note with regard to the success of our approach that the summation of the independently determined cavity, dipolar, and hydrogen bond contributions are within 0.5 kcal mol^{-1} of that indicated by the experimentally determined partition coefficient.

While there is insufficient information regarding the partitioning behaviour of the semiquinone anion $Ca^{\dot{-}}$ to carry out a complete description of its solvation, it is nevertheless possible to speculate on the changes in solvation incurred upon its formation. The most dramatic change upon reduction of the quinone is seen in the pK of the oxygen lone pairs. The pK of these lone pairs changes from −7.6 (*p*-benzoquinone) to approximately +5 (semiquinone) which according to Fig. 2, would imply an increase in the solvation contribution of the lone pairs from 0 to −3.16 kcal mol^{-1}. If we consider an estimated four hydrogen bonds to the semiquinone anion, their solvation would account for approximately −12 kcal mol^{-1}. With this in mind, the observed binding characteristics of the Q_B site of reaction centers in purple photosynthetic bacteria and PS II of plants, for the quinone, semiquinone and hydroquinone [27], can be explained by an initial low affinity of the lone pairs of the quinone carbonyl oxygens for the site, followed by a subsequently large increase in site affinity upon formation of the semiquinone anion and their subsequent decrease upon formation of the hydroquinone.

This paper has served to introduce a new strategy aimed at quantifying the thermodynamics of small molecule solvation. The method draws on the enormous compilation of partition coefficient already existing in the literature [12] coupled with conventional thermodynamic parameters. The method has, in principle, applicability to any homogeneous solvent environment, and shows promise for investigations of inhomogeneous environments characteristic of proteins and membrane systems.

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