Thermodynamic Studies of Hydrogen Bonding and Proton Transfer between Weak Acids and Bases in Nonaqueous Solvents as a Model for Acid-Base Reactions in Proteins

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Abstract: Dilute solutions of phenol and $CH_3(CH{=}CH)_5CH{=}NC_4H_9$ (compound **1**) in hydrocarbon solvents are subjected to decreasing temperature and their electronic absorption spectra recorded. Initially only the spectrum of the Schiff base, compound **1**, is present. As temperature is lowered, absorbance decreases and the spectrum of the H-bonded form of **1** appears. Continued lowering causes a decrease in absorption of the H-bonded form and a rise in absorption of the proton transferred form of **1**. The absorbance, and hence the concentration, of the species are measured quantitatively as a function of temperature, and these data are converted into $\Delta H°$ and $\Delta S°$ for each reaction step. The constants are used in $\Delta G°_T = \Delta H° - T\Delta S°$ to develop a description of events. In both reactions, values of both constants are negative. With respect to either equilibrium, the negative value of $\Delta H°$ displaces it to the right, and the negative value of $\Delta S°$ displaces it to the left. Hydrogen bonding is a *specific* reaction in which the intrinsic thermodynamic constants dominate. A large negative $\Delta S°$ results from the bimolecular, $2 \rightarrow 1$ reaction, but lowering temperature decreases the size of $-T\Delta S°$, forcing equilibrium to the right. The proton transfer step is more complex, but the size of the negative $\Delta H°$ appears to depend, in part, on the value of $\Delta S°$. This suggests that the sign and magnitude of the constants results from the action of the newly formed ion pair as it reorders its solvent cage. If in simple chemical systems, the value of the $-T\Delta S°$ can be decreased by decreasing temperature, it is deduced that in protein, $-T\Delta S°$ is decreased by decreasing the negative value of $\Delta S°$. This is accomplished when the protein folding process preorders amino acid side chains which are approximate to the reacting groups.

Introduction

In this article, a chemical system is described which employs UV-vis spectroscopy for investigating acid-base reactions; the system is then used to study protonation by a weak acid. Results from these experiments are employed to describe events taking place in the simple chemical system, and, finally, the description is used as a model for interpreting acid-base reactions in proteins. In earlier studies with retinal analogues related to visual pigments, we showed that the following Schiff base, $CH_3(CH{=}CH)_5C{-}H{=}NHC_4H_9$, compound **1**, has unique spectroscopic properties.[1] These properties were used to monitor the degree of temperature dependent interaction between compound **1** and the weak acid phenol. It was possible to measure formation of the resulting hydrogen bonded and proton transferred species as temperature is decreased. In these experiments, compound **1** and phenol were dissolved in a hydrocarbon and subjected to decreasing temperature, and spectra were recorded on a UV-vis spectrophotometer. The separated and corrected spectrum of each species, as determined in the present work, is shown in Figure 1. The three species obey the following thermal equilibria

$$SB + HOP \rightleftarrows SB{\cdots}HOP \rightleftarrows SBH^+{\cdots}{}^-OP \qquad (1)$$

SB, SB···HOP, and $SBH^+{\cdots}{}^-OP$ refer to compound **1**, its hydrogen bonded form, and its proton transferred form, respectively. Because the spectra are separable, a decrease in the absorbance of one and a simultaneous increase in another may be followed quantitatively. However each spectrum recorded on the spectrophotometer contains component absorbances of two species, and they are separated by application of the method for a two component mixture. The temperature dependent absorbances are reversible, and equilibrium constants were obtained for the hydrogen bonding process.[1] Logarithms of equilibrium constants were plotted against the reciprocal of recorded temperatures to obtain $\Delta H°_{HB}$ and $\Delta S°_{HB}$. However, proton transfer data were not of the quality required for quantitative treatment.

This is the only known UV-vis system directly related to retinal that is available for examining both hydrogen bonding and proton transfer; however, infrared spectroscopy has been employed by other workers.[2,3] In one of a number of articles by the Zundel group, a simple chemical system was described. It consisted of *N*-retinylidene-*n*-butylamine (NRBA) dissolved in methylcyclohexane (MCH) and a weak acid selected from a set of chlorinated phenols of varying acid strength.[2] When the mixture was examined by infrared spectroscopy, serial equilibria between three temperature dependent species were detected: NRBA, NRBA hydrogen bonded with chlorophenol, and the proton transferred species. Temperature dependent amounts of the three species were determined by measuring absorbance of the νO-H and νC=N$^+$H stretching frequencies. Under the conditions employed, neither phenol nor cresol could protonate NRBA, and an extrapolation procedure suggested protonation by these species is not possible. It was concluded that in rhodopsin, it is not possible for the unaided acidic hydroxyl group of tyrosine to protonate the Schiff base of retinal.[2] Other workers questioned how carboxyl groups of aspartic or glutamic acids could protonate the Schiff base of retinal in rhodopsin and bacteriorhodopsin.[3] Their system employed *N*-retinylidene-*tert*-butylamine and was followed by Fourier transform infrared spectroscopy. Protonation did not go to completion. As illustrated by these examples and references cited therein, acid-base reactions in visual proteins, and in fact in proteins in general, remain poorly understood.

Our initial study lead to these important results:[1] we showed experimentally that an acid as weak as phenol does protonate a weak base like NRBA under appropriate conditions. Secondly, we demonstrated the usefulness of compound **1** as a probe. By employing **1**, the hydrogen bonding and proton transfer processes in retinal related compounds can be studied directly through UV-vis spectroscopy; whereas formerly, only infrared methods were available. Finally, we developed much of the experimental protocol that is necessary for performing the quantitative work described here. The initial procedures did have limitations. For example, MCH, although a useful solvent, permits solute precipitation at low temperature, and, consequently, proton transfer could not be followed quantitatively. Furthermore, temperature control was unsatisfactory; cooling by pumping liquid nitrogen through the cell holder gave fluctuating temperatures. Fortunately,

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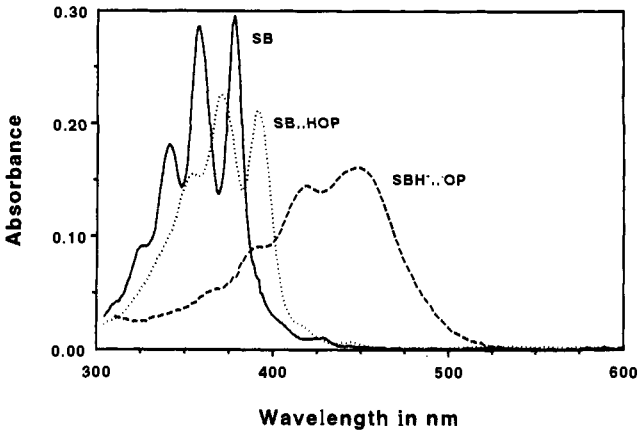

Figure 1. SB is spectrum of $CH_3(CH{=}CH)_5CH{=}NC_4H_9$ (compound 1). SB···HOP is corrected spectrum of compound 1 hydrogen bonded with phenol. $SB^+\cdots{}^-OP$ is corrected spectrum of compound 1 protonated by phenol.

most of the experimental problems encountered in the initial work have been solved. Thermodynamic constants have now been determined for both processes, and they are used to describe both reaction steps. Additionally, two previously described concepts are employed to help explain acid–base reactions in simple chemical systems and in proteins. One concept describes contributions of entropy in protein mediated reactions;[4-6] the other describes contributions of both *specific* and *nonspecific* interactions to the thermodynamic constants for each reaction step.[7]

Experimental Section

Solvents. All purchased reagents and solvents were purified by standard organic laboratory procedures.[1,8] Because of the low temperatures required for thermal equilibrium studies, the choice of solvent presented considerable difficulty. Methylcyclohexane and 3-methylpentane (3MP) were used separately as solvents with varying success; however recently, a 1:1 by volume mixture gave excellent results. Solutes precipitate from 3MP at a higher temperature than from MCH, and, for unknown reasons, MCH produces erratic spectroscopic base lines. Employing the mixture we find that (i) precipitation does not occur, (ii) base line corrections are greatly reduced, and (iii) glass formation does not occur until much lower temperatures.

When a hydrocarbon solution is cooled, its concentration increases because solvent volume contracts. Because concentration is used in calculation of equilibrium constants for the van't Hoff plot, the coefficient of contraction (expansion) is required. Values may be found in the literature for MCH and 3MP but not for the mixture. This was determined with a pycnometer by following the method of Lipkin.[9] The density of the 1:1 volume mixture is 0.7168 g/mL at 293 K, and the coefficient of expansion is 0.000923 g/mL/dgr.

Preparation and Purification of Aldehyde and Schiff Base. 2,4,6,8,10-Dodecapentenal was prepared from crotonaldehyde by the modification of the method of Blout.[10] Crotonaldehyde is treated with a catalytic mixture of piperidine and glacial acetic acid and heated to 97 °C for 45 min under diminished red light and nitrogen atmosphere. After a second addition of catalyst and further heating, the unreacted aldehyde and water are removed by vacuum distillation, and the residue is extracted with ether. The filtrate is washed with water and dried over $MgSO_4$. After ether removal, the residue is vacuum distilled. The product is purified first by column chromatography and then by several recrystallizations from methanol. Feathery yellow needles were obtained that melted at 164–167 °C. HPLC on a silica gel was used to assay purity of the aldehyde. Compound 1 was prepared according to our published methods.[1]

Obtaining Variable Low Temperature UV-vis Spectra. Part of the uniqueness of this work rests on the discovery that compound 1, when dissolved in hydrocarbon, treated with a weakly acidic donor, and subjected to progressively lower temperature, shows three separable, sequentially appearing spectra (Figure 1). The key to these experiments is to achieve and hold constant a number of low temperatures so spectra can be recorded. This was done by employing a variable temperature liquid nitrogen cryostat from Oxford Instruments. The cryostat, which accepts a single, standard 1-cm path length sample cell, is fitted into a Hitachi U-3210 double beam spectrophotometer. The spectrophotometer was modified by Hitachi as follows: a special cell compartment cover was fitted which allowed insertion of the cryostat, and the floor of the compartment was modified to accept and position the cryostat. With this arrangement, spectral measurements were made at intervals of 5 °C. Past experience showed that a 1-cm path length cell requires concentrations of 1 to be in the range of $5–9 \times 10^{-6}$ M to achieve a desirable absorbance range. A phenol concentration of about $5–9 \times 10^{-4}$ M is found to be best. This concentration is small enough so that only about 3% of the SB is converted to SB···HOP at room temperature, and the proton transfer reaction is 90% completed at 158 K.

Measuring Changes in Absorbance. The spectrophotometer is interfaced with an IBM Model 50z personal computer system driven by software necessary for spectral curve transfer and manipulation. Compound 1 is dissolved in MCH to prepare a standard solution, and its spectrum is determined from 800–250 nm. The exact concentration is determined from absorbance of the 373-nm band whose extinction coefficient is 128 000. A hard copy of the spectrum is made on the instrument, and a copy is transferred to the hard disk of the computer. A standard solution of phenol is prepared, and concentration is determined spectrophotometrically at 270 nm $\epsilon = 237.4$. The two solutions are mixed by standard volumetric techniques to obtain the desired concentrations. The sample cell is loaded and placed in the cryostat, and the spectrum is again determined and stored. All spectra are scanned from 800–250 nm, and if they are to be retained, they are stored on the hard disk.

Liquid nitrogen is loaded in the cryostat, and the desired value is set on the temperature controller. In the hydrogen bonding step, absorbance at approximately 389 nm is followed in a time scan mode to determine when equilibrium is reached at a given temperature. This usually takes upward of 30–40 min. First the spectrum is recorded on paper, and it is then stored in the computer, and, at the same time, the temperature is recorded. The process is repeated over the desired temperature range.

Lowering temperature causes these changes: (i) an alteration of the base line; (ii) a shift of the reaction toward hydrogen bonded formation, changing the value of the equilibrium constant; (iii) a contraction of solvent volume, increasing solute concentration; (iv) and a migration of λ_{max} values to longer wavelengths. All of these cause spectral changes.

Spectral Corrections. Base Line and Spectral Migration Corrections. Spectra are recorded from 800–250 nm, and base line corrections are made in two steps. As temperature is lowered, absorbance at 800 nm does increases from zero; and proceeding from 800 to 600 nm it follows a straight line with an upward slope. First the increased absorbance at 800 nm is either subtracted from the entire spectrum or from the absorbance at the A′ band (at approximately 389 nm). Next the error caused by the upward slope is corrected by subtraction. Correction can be made either at 389 nm or throughout the spectrum. Correction at 389 nm is done by measuring the adjusted absorbance at 600 and designating it as "*a*" and designating the absorbance at 389 nm as "*b*". The corrected value at *b* is found by the ratio $b = a(800 - \lambda_{max})/(800 - 600)$. Because the A′ band migrates to longer wavelengths as temperature is lowered, its exact location must be found for each temperature. We showed previously that absorption bands of linear polyenes move at approximately the same rate, 3.7 cm^{-1} dgr^{-1}, as temperature is lowered.[1] Applying these corrections gives absorbance of the A′ band as though it had been recorded at the given temperature in a true double beam mode, and it is designated A_t.

Solvent Contraction Correction. Since liquid density is temperature dependent, and since for a given amount of solute, its concentration is dependent on volume, absorbance depends on temperature. The aim of this correction is to compare all reactions at constant volume. The absorbance of the A′ band, A_t, at temperature *t*, is adjusted by a volume correction term to yield an absorbance term, A_T, which allows comparison of absorbance at a standard comparison temperature (293 K). In the conversion of A_t to A_T, the following quantities are available: A_t, defined above; D_{293} the density of solvent at 293 K; *F* the coefficient of expansion or contraction (0.000 923 g/mL/dgr for the mixed solvent); and C_{293} the concentration of compound 1 at 293 K. Absorbance at the recording temperature is given as $A_t = \epsilon C_t l = \epsilon m l / V_t$, where *m* = moles of solute,

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V = volume of solution, l = path length of cuvette, and ϵ = extinction coefficient. In like manner, absorbance at 293 K is given as $A_T = \epsilon ml/V_{293}$. $A_T/A_t = (\epsilon ml/V_{293})/(\epsilon ml/V_t)$, and $A_T = A_t(V_t/V_{293})$. However $V_t = \theta V_{293}$, where $\theta = D_{293}/D_t$ and $D_t = D_{293} + F(293 - t)$. When the volume correction is made $A_T = A_t(V_{293}\theta/V_{293})$, or $A_T = \theta A_t$. Thus A_T is the absorbance of the A′ band which is first measured at temperature t, corrected for base line, and then corrected to the value it would have if it had been recorded at 293 K.

Correction for Second Component. Absorbance at 389 nm comes from two components, SB and SB···HOP. As temperature is decreased and SB is converted to SB···HOP, the amount of SB decreases, and its absorbance at 389 nm decreases. Since absorbance of SB···HOP is being measured, absorbance of SB is subtracted as follows. Terms are defined, and all are adjusted to their value at 293 K. A_T = absorbance of A′ band as given above; $A_{T_{HB}}$ = that portion of A_T attributable to SB···HOP; A_f = maximum value of $A_{T_{HB}}$; b_o = the maximum absorbance of SB; and b_T = that portion of A_T attributable to Schiff base. Absorbance at 389 nm and at 293 K is $A_T = b_T + A_{T_{HB}}$, and this is rearranged

$$A_{T_{HB}} = A_T - b_T \tag{2}$$

The adjusted fraction of SB···HOP formed is $A_{T_{HB}}/A_f$, and the adjusted fraction of SB reacted is $(b_o - b_T)/b_o$. The values of these two fractions are equal and equated, $A_{T_{HB}}/A_f = (b_o - b_T)/b_o$, and solved for b_T

$$b_T = b_o(A_f - A_{T_{HB}})/A_f \tag{3}$$

Substitute eq 3 into eq 2 and solve for $A_{T_{HB}}$

$$A_{T_{HB}} = A_f(A_T - b_o)/(A_f - b_o) \tag{4}$$

$A_{T_{HB}}$ and A_f are used for calculating equilibrium constants.

Equilibrium and Thermodynamic Constants. Calculation of Equilibrium Constants. The equilibrium constant for the hydrogen bond interaction at temperature t is

$$K_{eq_t} = \frac{[\text{SB···HOP}]_t}{[\text{SB}]_t[\text{HOP}]_t} \tag{5}$$

The concentrations in the equilibrium expression are evaluated by first measuring the fractions of Schiff base and hydrogen bonded Schiff base at temperature t. The fraction hydrogen bonded Schiff base is $f_{(SB···HOP)_t} = A_{T_{HB}}/A_t$, and the fraction of Schiff base remaining is $f_{SB_t} = 1 - f_{(SB···HOP)_t}$. Equilibrium concentrations at temperature t are used in expression K_{eq_t}. They are calculated from the initial concentrations C by employing the fractions given above and by applying a solvent expansion parameter ρ ($\rho = 1/\theta$, where θ was defined previously). Starting with the hydrogen bonded Schiff base concentration at temperature t, $[\text{SB···HOP}]_t = \rho C_{SB} f_{(SB···HOP)_t}$; next the concentration of Schiff is $[\text{SB}]_t = \rho C_{SB} f_{SB_t}$; and finally the concentration of phenol is $[\text{HOP}]_t = \rho(C_{HOP} - C_{SB} f_{(SB···HOP)_t})$. These concentrations are substituted in eq 5 and rearranged to give eq 6.

$$K_{eq_t} = \frac{1}{\left(\dfrac{f_{SB_t}}{f_{(SB···HOP)t}}\right)\rho(C_{HOP} - C_{SB} f_{(SB···HOP)_t})} \tag{6}$$

A similar but not identical derivation is repeated for determination of equilibrium constants in the proton transfer reaction. In this case the equilibrium is as follows:

$$K_{eq_t} = \frac{[\text{SBH}^+\cdots^-\text{OP}]_t}{[\text{SB···HOP}]_t}$$

Initially, in the proton transfer process, absorbance changes were monitored by following the 450-nm band to determine the fraction reacted. Then it was observed that the spectrum of the proton transferred species continues to change after protonation is completed. Consequently, loss of absorbance at 350 nm was used to measure this process.

Results

Calculation of Thermodynamic Constants. The logarithms of K_{eq} were plotted versus $1/T$ in van't Hoff plots to obtain the ΔH°_{HB} and ΔS°_{HB} for the hydrogen bonding process and ΔH°_{PT} and ΔS°_{PT} for the proton transfer process. Figures 2 and 3 show representative van't Hoff plots for hydrogen bonding and proton transfer, respectively, and the lines have the following R values: 0.995 and 0.993. Table I gives resulting representative values of ΔH°, ΔS°, and ΔG° from several experimental determinations. This is the first reporting of thermodynamic constants for the proton transfer step for the phenol-**1** system. The constants for

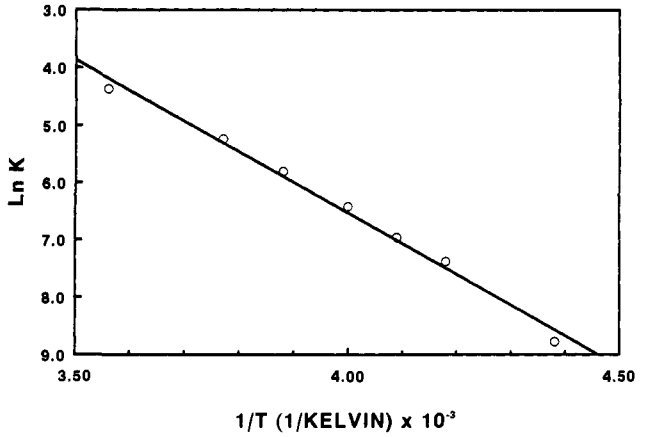

Figure 2. Representative van't Hoff plot for the hydrogen bonding step of the phenol–compound **1** system in 1:1 mixture of MCH and 3MP.

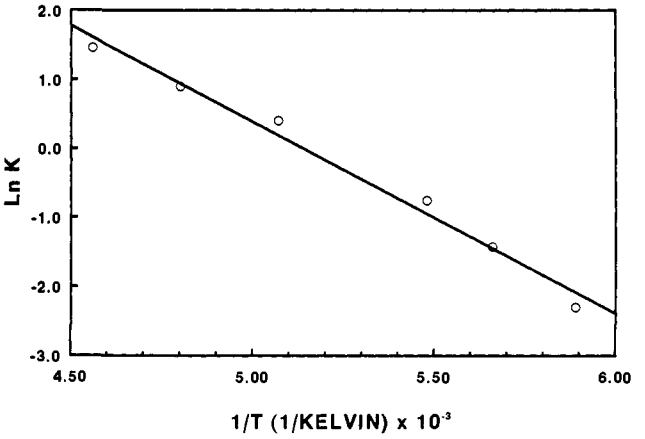

Figure 3. Representative van't Hoff plot for the proton transfer step of the phenol–compound **1** system in 1:1 mixture of MCH and 3MP.

Table I. Thermodynamic Constants for H-Bonding and Proton Transfer: $CH_3(CH{=}CH)_5CH{=}NC_4H_9$ and Phenol at 310 K[a]

solvent	ΔH°_{HB}	ΔS°_{HB}	ΔG°_{HB}	ΔH°_{PT}	ΔS°_{PT}	ΔG°_{PT}	ΔG°_{T}
MCH	−8.6	−21.7	−1.9	−5.7	−31.8	+4.2	+2.3
MCH	−8.7	−22.8	−1.6	−5.5	−28.5	+3.3	+1.7
MIX	−9.2	−24.0	−1.8	−5.7	−29.5	+3.4	+1.6
av	−8.8	−22.8	−1.8	−5.6	−29.9	+3.6	+1.9

[a] ΔH°, ΔG° kcal mol^{-1}; ΔS° kcal mol^{-1} K^{-1}. MCH = methylcyclohexane; MIX = 1:1 (v/v) mixture of MCH and 3-methylpentane.

phenol–NRBA were not accessible through infrared spectroscopy.[2] The constants for the hydrogen bonding process compare favorably with those reported in the literature. The ΔH° for phenol–pyridine in CCl_4, measured by calorimetry, is −7.61 kcal mol^{-1}.[11] ΔH° and ΔS° values determined by infrared spectroscopy for phenol–pyridine are −6.5 kcal mol^{-1} and −14 cal mol^{-1} K^{-1}, respectively, in CCl_4;[12] and −8.96 kcal mol^{-1} and −14.8 cal mol^{-1} K^{-1} respectively in cyclohexane.[13]

Discussion

Protonation: A Two-Step Process. The results we have found through UV–vis spectroscopy are in agreement with those from infrared spectroscopy[2] and show that simple chemical protonation in the phenol–**1** system is a two-step process. First there is hydrogen bonding and then proton transfer from phenol to **1**. The two steps are represented by eq 1, and, as shown in Figure 1, each

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newly formed species has its individual spectrum. The values of $\Delta H°$ and $\Delta S°$ are negative for both reaction steps, an important characteristic found in many nonaqueous acid–base reactions.[14,15] The interaction of these negative terms is explained through use of eq 7.

$$\Delta G_T^\circ = \Delta H° - T\Delta S° \quad (7)$$

In each reaction step, the negative $\Delta H°$ acts to displace the equilibrium to the right. On the other hand, a negative value of $\Delta S°$ counteracts the action of $\Delta H°$ and displaces the equilibrium to the left. In each reaction, temperature is the variable and determines whether the equilibrium is shifted to the right or left. As temperature decreases, counter balancing by $T\Delta S°$ decreases and equilibrium is displaced to the right.

Our results are in agreement with those of Zundel, who employed a set of chlorphenols and NRBA in MCH.[2] Although the two systems have similarities, they differ in important respects. Because our system employs UV–vis spectroscopy, the molar extinction of compound **1** dictates a concentration of about 5–9 $\times 10^{-6}$ M, and phenol is required at about 5–9 $\times 10^{-4}$ M. In Zundel's system, because measurements were made by infrared spectroscopy, the required concentration of NRBA was much higher, about 2×10^{-2} M; and of additional importance, NRBA and the chlorophenol were present in equal molar amounts, giving a 1:1 solution in MCH. Thus in their samples the Schiff base was 10000 times more concentrated, the mixture was 1:1, and their was no excess of phenol.

Hydrogen Bonding in Simple Chemical System. In Zundel's analysis of the chlorophenol–NRBA system, he also found that acid–base reactions are two-step processes, and he postulated that each step includes contributions from *specific* and *nonspecific* components.[7] For example, bringing together a chlorophenol and NRBA to form the hydrogen bonded species is a *specific* reaction. However, the manner in which the newly formed species interacts with its surrounding solvent environment is a *nonspecific* reaction. Thermodynamic constants for each reaction step were separated into two component sets; one *intrinsic*, resulting from *specific* reactions, and the other *extrinsic*, resulting from *nonspecific* electrostatic interactions between solute and solvent. Thus $\Delta H° = \Delta H_i^\circ + \Delta H_e^\circ$, and $\Delta S° = \Delta S_i^\circ + \Delta S_e^\circ$. When permittivity of the solution environment is one, $\epsilon = 1$, the *extrinsic* constants, ΔH_e° and ΔS_e°, have values of zero; the *intrinsic* constants ΔH_i° and ΔS_i° become the actual thermodynamic constants. When solvent permittivity increases, the *extrinsic* constants become increasingly more negative. In any given reaction step the question is which set of constants, intrinsic or extrinsic, makes the largest contribution to the observed values. In the simple chemical systems represented here, hydrogen bonding is the *specific* reaction, and the intrinsic set makes the major contribution to ΔH_{HB}° and ΔS_{HB}°. For the H-bonded species, *nonspecific* interaction with solution environment is not as great as it is for the ion pair, consequently it makes a smaller contribution. The order of contribution is reversed for the proton transfer.

The ΔH_{HB}° of −8.8 kcal mol^{-1}, given in Table I, is the loss of energy caused principally by the *specific* reaction, the hydrogen bond formation between the phenolic hydrogen and the lone electron pair on the nitrogen of compound **1**. Its value is comparable with, but somewhat larger than, those cited above.[11-13] The small differences among thermodynamic constants reported in the literature, and also those reported here, are ascribed to two sources. One source is the variety of bases and phenols used experimentally, since they would be expected to give rise to differing intrinsic thermodynamic constants.[2,7,15] A second source is the variety of experimental solution environments, since they also would be expected to give rise to differing extrinsic constants. Solute–solvent interaction is explored in detail later.

The reason for the large negative entropy change for the hydrogen bonding step is qualitatively explained by the work of Page and Jencks.[5] In a *specific*, bimolecular, 2 → 1 reaction, where two independent solution species unite to form one, loss of translational and rotational freedom in the product results in a large entropy loss. They concluded the observed entropy loss for many bimolecular reactions in solution could have negative values as large as −35 cal mol^{-1} K^{-1}. In our phenol–**1** system, hydrogen bond formation is a *specific*, bimolecular, 2 → 1 reaction; there is loss of translational and rotational freedom in the product which gives rise to the large negative ΔS_{HB}° of −22.8 cal mol^{-1} K^{-1}. The entropy loss results primarily from the *specific* reaction, and it receives its major contribution from changes in intrinsic entropy. Extrinsic entropy changes are discussed later.

Effect of Temperature. The role of temperature in controlling the hydrogen bonding step of acid–base reactions is shown as follows. Employing the thermodynamic values from row 1, Table I, i.e., concentrations of [SB] = 7.79×10^{-6} M and [HOP] = 8.66×10^{-4} M, temperature = 298 K, and the standard thermodynamic relationships, the conversion to SB···HOP is calculated to be only 3.1%. Values of SB conversion of 20, 50, and 80% occur at 261, 241, and 224 K. Thus as temperature is decreased, equilibrium is shifted to the right, and this is explained in eq 7. For a specifically defined reaction system, $\Delta H°$ and $\Delta S°$ are constant, and, according to eq 7, ΔG_T° varies with temperature. The degree of hydrogen bonding is driven only by the magnitude of the negative enthalpy. Because $\Delta S°$ in these reactions is always negative, the $-T\Delta S°$ term contributes to the reversal of the reaction. However $-T\Delta S°$ makes a smaller contribution as temperature is decreased. Thus in the laboratory, hydrogen bonding can be forced to completion by lowering temperature. It is also clear from eq 7 that if ΔS_{HB}° assumes a smaller negative value, the effect would be equivalent to lowering temperature.

Does the self-association of phenol have a serious effect on the phenol–**1** equilibrium. The self-association constant of phenol in CCl_4 was found to be 2.26 M^{-1} at 294.5 K,[16] and $\Delta H° = -4350$ cal/mol.[17] Using this information and the concentrations given above, $\Delta G°$ values can be calculated and compared. The values of ΔG_{298}° for association in the phenol–**1** system and for self-association in phenol are −2130 and −416 cal/mol, respectively. Comparison of percent completion of the reaction is more meaningful. As given above, the phenol–**1** reaction is 80% complete at 244 K, whereas phenol self-association is only 2.9% complete. The phenol self-association reaction does not greatly interfere with hydrogen bonding in the phenol–**1** reaction.

Proton Transfer in Simple Chemical Systems. Proton transfer is an equilibrium between the tautomeric forms[14] in eq 1, SB···HOP ⇄ $SBH^+\cdots{}^-OP$, and interconversion is a 1 → 1 reaction. The process is often described by a double well potential in which each tautomeric species exists separately in one of the wells.[2,14,18] According to Zundel,[7] "The mean energy difference of the two wells of a double minimum proton potential in an AH···B ⇌ $A^-\cdots H^+B$ hydrogen bond is classically determined by $\Delta H°$". Table I gives this enthalpy change. For proton transfer, ΔH_{PT}° is a much smaller negative quantity than for H-bonding, −5.6 versus −8.8 kcal mol^{-1}; but surprisingly the entropy change is a larger negative value, −29.9 versus −22.8 cal mol^{-1} K^{-1}. The overall proton transfer process is actually comprised of at least three *specific* events, i.e., H–O bond breaking, H–N bond formation and charge separation, and, most importantly, a *nonspecific* electrostatic interaction between the newly formed ion pair and solvent dipoles. Therefore ΔH_{PT}° is a complex term; it is the sum of the enthalpies of all the listed events. From inspection of available results, it appears that $\Delta H_{PT_i}^\circ$ (intrinsic enthalpy) may be a small negative number of 1 kcal mol^{-1} or less. As in the case of hydrogen bond formation, lowering temperature drives proton transfer reactions to completion; therefore, ΔH_{PT}° must be a negative value.

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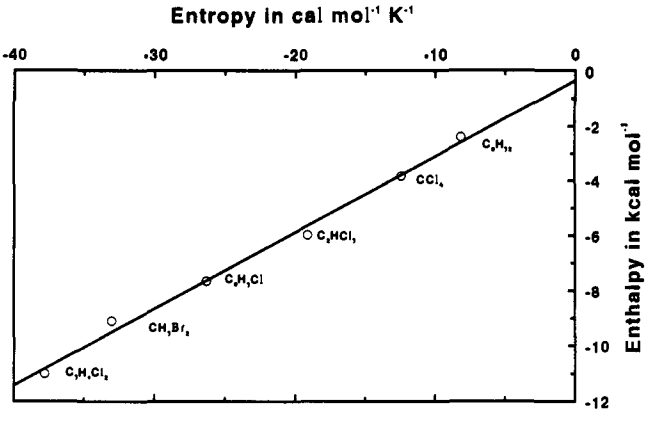

Figure 4. Plot of enthalpy and entropy values for 2,3,5-trichlorophenol–octylamine in solvents of increasing permittivity. Data taken from ref 7.

The size of the entropy change discloses important information. For this simple chemical, intramolecular, $1 \rightarrow 1$ process, the loss of degrees of freedom is expected to be much smaller than for intramolecular hydrogen bond formation. However, the reverse is true; ΔS°_{PT} is a larger negative value than ΔS°_{HB}, −29.9 versus −22.8 cal mol^{-1} K^{-1}. Rather than arising from *specific* reactions, the large negative ΔS°_{PT} must arise from *nonspecific* reactions in which a solute ion pair orders adjacent solvent molecules into a solvent cage. Initially, in our system, MCH was viewed as solvent and phenol as the acid reactant, but the system is more complicated than originally conceived. MCH, a hydrocarbon of low dielectric constant, was chosen because it would have little or no effect on the reaction process. This is true, but phenol, which is present at 100 times the concentration of the Schiff base, plays two roles. It engages in a *specific* 1:1 reaction with compound **1** to form SB···HOP, and because of it high concentration it behaves *nonspecifically* as an active solvent component. Thus phenol and not hydrocarbon molecules immediately surround the solute ion pair and interact electrostatically at that site. In this way, the ion pair reorders phenol molecules into a cage and greatly increases the negative value of ΔS°_{PT}. The ensuing interaction between the ion pair and the electrostatic field of the newly formed solvent cage also increases the negative value of ΔH°_{PT}. This phenomenon was substantiated experimentally by Zundel.[7] ΔH°_{PT} and ΔS°_{PT} values were obtained for the 1:1 complex of 2,3,4-trichlorophenol–octylamine in six chlorohydrocarbon solvents whose permittivity increased in the following order: C_5H_{12}, CCl_4, C_2HCl_3, C_4H_9Cl, CH_2Br_2, $C_2H_4Cl_2$. In five solvents (less CH_2Br_2), the negative value of ΔH°_{PT} increased linearly as the Onsager parameter[19] of the solvent increased. Thus increases in solvent permittivity induce increased extrinsic interaction between the solute ion pair and solvent dipoles.

The foregoing suggests a possible linear relationship between the term for ordering solvent dipoles, $-T\Delta S^\circ_{PT}$, and the interaction between the ion pair and the field strength of the ordered cage, ΔH°_{PT}. We tested this by plotting Zundel's data. In Figure 4, ΔH°_{PT} is plotted versus ΔS°_{PT}, and a straight line correspondence is found (R value = 0.997). (Interestingly, Zundel's data for CH_2Br_2 also falls on this straight line.) Under the solvent conditions of our experiment, the ion pair–solvent interaction is most likely a dipole–dipole interaction. Since dipoles are vectors, both charge and orientation are important in determining the resultant moment. Thus in order to gain maximum interaction, a realigning (reordering) of solvent dipoles is required; i.e., any increase in the negative value of ΔH is only gained at the expense of an increased negative value of ΔS°. Reasoning with eq 7, we see that an attempt to achieve a larger negative value of ΔG° through increasing the negative value of ΔH° is offset by a larger negative value of ΔS°. However, again rationalizing with eq 7, it is possible to achieve proton transfer in the laboratory by lowering temperature and thereby decreasing the value of the solvent ordering term $-T\Delta S^\circ_{PT}$.

The effect of solvent permittivity and temperature on $-T\Delta S^\circ$ can be visualized by further examination of Zundel's results.[7] Employing their thermodynamic constants for the 2,3,4-trichlorophenol–octylamine system in solvents of increasing permittivity (C_5H_{12}, CCl_4, C_2HCl_3, C_4H_9Cl, CH_2Br_2, $C_2H_4Cl_2$), they calculated ΔG_{293} values to be −7.9, −187, −384, +56, +75, +589 cal mol^{-1}. Thus at 293 K, increasing solvent permittivity first causes the ΔG° to increase negatively and then retreat to greater positive values. At 293 K, the equilibrium is displaced to the right in the first three solvents and then to the left in the next three solvents because of the increasing negative value of ΔS°. We calculated the same series at 193 K and found ΔG°_{193} values to be −0.821, −1.43, −2.29, −2.57, −3.70, −2.71 kcal mol^{-1}. At this temperature all reactions are displaced to the right.

Hydrogen Bonding and Proton Transfer in Protein. In preceding sections, the phenol–**1** system was compared with similar acid–base systems described by other workers. Insight gained is now applied to proteins. The following postulates apply to proteins in living systems: (i) Protonation takes place between weakly acidic and basic side chains of amino acid residues. (ii) As in simple chemical systems, protonation in proteins proceeds through two separate steps, hydrogen bonding and proton transfer, and these steps include both *specific* and *nonspecific* components. (iii) In the biological setting, the temperature in the $-T\Delta S^\circ$ term is approximately constant. (iv) The $\Delta H^\circ_{HP_i}$ and $\Delta H^\circ_{PT_i}$, which are attributable to *specific* reactions, are expected to be approximately the same as in the simple system. Consequently, only changes in ΔS° are available for controlling the equilibrium of a reaction.

Even though hydrogen bonding in a protein is a bimolecular, $2 \rightarrow 1$ process, protein folding has brought the interacting side chains in close approximation and fixed them in space. A similar situation exists in enzymes, a topic already examined by Page and Jencks[5,6] and described above. We modify and employ this hypothesis to fit the hydrogen bonding step of acid–base reactions in proteins. In protein, acid and base functional groups are held in fixed position by being part of the side chains of amino acids which themselves are held in fixed position as part of the fixed tertiary structure of the protein. Hydrogen bonding between approximal groups occurs with little loss of intrinsic entropy, and the ΔS°_{HB} will be a considerably smaller negative value than the −22.8 cal mol^{-1} K^{-1} given in Table I. Consequently, the $-T\Delta S^\circ_{HB}$ term makes a smaller positive contribution to ΔG°_{HB}, and the hydrogen bonding reaction is displaced to the right.

A description of the factors controlling proton transfer in proteins differs from that of hydrogen bonding, and it is based on the following postulates: (i) The side chains of amino acids engaging in acid–base reaction are already joined by hydrogen bonding. (ii) Since the protein has already acquired its tertiary structure, amino acid side chains surrounding the acid–base pair have also acquired their specific and fixed orientation. (iii) The ensuing proton transfer consists of both *specific* and *nonspecific* interactions and both make contributions to intrinsic and extrinsic changes in enthalpy and entropy. (iv) The overall *specific* reaction found in proteins is expected to be similar to that of simple chemical systems and proceeds through an equivalent set of events including H–O bond breaking, H–N bond making, and charge separation. As in the simple chemical process, tautomerization in the protein is expected to have a small value of intrinsic entropy ΔS°_i. Tautomerization in the protein is also expected to have a negative and small value of intrinsic ΔH°_i.

There is also a *nonspecific* electrostatic interaction between the newly formed ion pair and the dipoles of approximate amino acid side chains; it can be understood by comparing it with the *nonspecific* reaction in a simple chemical system. First the ion pair reorders solvent molecules into a solvent cage and then interacts with the solvent cage. Here the extrinsic thermodynamic constants ΔH°_e and ΔS°_e become important. Insight is gained from the following results. When 2,3,4-trichlorophenol–octylamine was dissolved in solvents of increasing permittivity, it was shown that the negative value of ΔH°_{PT} increases linearly with increases in

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the negative value of ΔS°_{PT}.[7] This was explained through electrostatic interaction between the solute ion pair and the solvent dipole. For solvent molecules with negligible or zero dipole moment, the ion pair engages in little or no reordering of solvent molecules; however, the ion pair does engage in a large reordering of solvents with large dipoles. As a corollary, the larger the dipole moment of the solvent, the greater the electrostatic field strength of the rearranged solvent, and the larger the interaction, ΔH°_{PT}, between the ion pair and solvent cage. When applying this to proteins, it is recalled that the two acid-base side chains are already arranged in hydrogen bond formation. Furthermore, the amino acid side chains immediately adjacent to the ion pair are also fixed in space through protein folding. In this particular case, side chains are spatially arranged so their dipoles engage in supportive electrostatic interaction. Since there is little reordering, ΔS°_{PT} for the process is negligible or a small negative value. However, these highly ordered side chains now engage in a strong electrostatic interaction with the ion pair, contributing a substantial negative value to ΔH°_{PT}. The small negative ΔS°_{PT} coupled with the large negative ΔH°_{PT}, when fitted into eq 7, yields a large negative ΔG°_{PT}. Equilibrium is displaced to the right, driving the proton transfer process to completion.

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Biocatalytic Synthesis of Aromatics from D-Glucose: The Role of Transketolase

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Abstract: The percentage of D-glucose that microbes such as *Escherichia coli* convert into aromatics is one of the key considerations that will determine the long-term utility of such biocatalytic syntheses. Considerable research has focused on improving percent conversions by increasing the in vivo catalytic activity of the first enzyme involved in aromatic biosynthesis, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) synthase. This report details a different strategy based on increasing the in vivo catalytic activity of the enzyme transketolase when DAHP synthase catalytic activity is amplified. A genetic locus (*tkt*) encoding transketolase was used to vary the catalytic activity of transketolase in *E. coli*. The catalytic activity of DAHP synthase was manipulated with an *aroG* gene encoding an isozyme of DAHP synthase that was insensitive to feedback inhibition. Increases in DAHP synthase catalytic activity ultimately reached a point where no further improvements in aromatic biosynthesis were observed. At this limiting level of aromatic biosynthesis, amplification of transketolase catalytic levels achieved an additional 2-fold increase in the percentage of D-glucose siphoned into aromatic biosynthesis.

Chemists have long been aware of the potential advantages of using simple carbohydrates such as D-glucose as synthetic starting materials.[1] Carbohydrates are relatively inexpensive and provide an almost boundless pool of chiral building blocks. Unfortunately, conversion of carbohydrates into noncarbohydrate products often requires long syntheses. Only a small amount of product is typically produced at the end of these synthetic efforts. An alternate approach to small-molecule synthesis entails genetic alteration of microbes to create a new organism capable of converting carbohydrates such as D-glucose into a noncarbohydrate product. Genetic modification of an organism requires as many tedious manipulations to create the new organism as chemical synthesis does to convert carbohydrate starting material into the desired product. The key advantage of the genetic approach is that a catalytic entity is created.

Perhaps the most spectacular example of the use of simple carbohydrates as starting materials in the synthesis of natural products is the conversion of D-glucose (**1**) by plants and microbes into aromatic amino acids and related metabolites (Scheme I).[2] Tremendous strides have been taken in the biocatalytic synthesis of L-phenylalanine (**2**) and L-tryptophan (**3**) from D-glucose.[3] These aromatic amino acids along with L-tyrosine (**4**) are used as human and animal dietary supplements. Of equal importance are the molecules that can be chemically or biocatalytically derived from the aromatic amino acids. L-Phenylalanine is transformed into the artificial sweetener aspartame (**5**) by traditional chemical methodology[4] while L-tryptophan and L-tyrosine can be converted to indigo (**6**) and eumelanin (**7**), respectively, by suitably constructed *Escherichia coli*.[5,6] Indigo imparts denim jeans with their distinctive, faded-blue coloration. Melanins provide the basic color pigmentation in mammals and birds while classes of melanin such as eumelanin possess unique UV-absorbing characteristics.[7]

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