of the sterically crowded Z isomer. To account for this dramatic shift in stereochemistry, we propose an intramolecular, through-space, charge-transfer complex[12] involving one of the tri-*n*-butylphosphonium groups in I and the π-electrons of the aromatic ring of the aldehyde.

If one envisions the classical Wittig mechanistic sequence[4a] (Scheme I) of the formation of betaine IV followed by cyclization to oxaphosphetane, then it can be seen that there is a choice as to which of the two diastereotopic phosphonium centers will be included in the ring. A concern in the classical Wittig reaction mechanism is the distribution of diastereomeric betaines. In this study diastereomeric betaines are not possible. Yet, on the basis of current mechanistic proposals, formation of the transoxaphosphetane V minimizes steric interaction between the R group of the aldehyde and the second phosphonium center of the ylide, thus resulting in the predominant formation of the E olefin. This line of reasoning is consistent with the E stereochemistry observed when aliphatic and alicyclic aldehydes react with I. However, aromatic aldehydes exhibit Z selectivity. Therefore, the more sterically hindered cis oxaphosphetane VI must predominate. The proposed through-space, charge-transfer complex between the aromatic ring and the positively charged phosphonium center accounts for this dramatic shift in stereochemistry. Therefore, Z stereoselectivity results when rotation of the betaine simultaneously allows for (a) the proposed through-space, charge-transfer complex and (b) the participation of the other phosphonium center in ring closure to oxaphosphetane.

The coplanar cycloaddition mechanism proposed by Giese and co-workers[3] for stabilized ylides can be similarly modified to account for the observed variations in stereochemistry. Accordingly, the effects of (a) steric crowding at phosphorus and (b) increased nucleophilicity of the ylide due to alkyl substituents result in formation of the carbon–carbon bond in advance of the phosphorus–oxygen bond; thus, steric interactions between substituents on the ylide carbon and carbonyl carbon favor the trans oxaphosphetane. This mechanistic interpretation predicts the

STABILIZED YLIDE

TRANSITION STATES
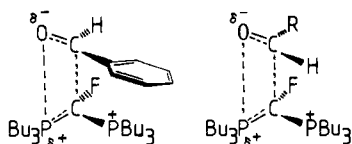
formation of the observed E isomer when utilizing aliphatic and alicyclic aldehydes. Invoking the proposed through-space, charge-transfer complex, the aromatic ring of the aldehyde interacts with one of the phosphonium centers as the ylide and carbonyl approach each other in a coplanar manner. Therefore, as the carbon–carbon bond forms, the aromatic ring and a phosphonium center are cis.

The proposed through-space, charge-transfer complex appears to be sensitive only to electron-withdrawing substituents on the aromatic ring. This causes the proposed interaction to weaken, allowing for the formation of significantly more of the E isomer; thus the steric and electronic nature of the ylide become dominant. Substituents in the ortho position on the ring enhance the Z selectivity. This Z selectivity appears to be unique, and we have no explanation for it at this time.

Operational details of the experimental procedure are outlined below for the preparation of 1-fluoro-2-phenylethene.

A 250-mL three-necked flask, equipped with magnetic stir bar, rubber septum, and nitrogen tee, was charged with 0.090 mol (18.2 g, 22.4 mL) of tri-*n*-butylphosphine and 30 mL of methylene chloride. The solution was cooled in an ice bath, and 0.030 mol (4.1 g, 2.8 mL) of trichlorofluoromethane was added in one portion via syringe. The resultant mixture was stirred at 0 °C for 1 h

and then at room temperature for 3 h.[9] To this phosphoranium salt solution was added 0.024 mol (2.5 g, 2.4 mL) of freshly distilled benzaldehyde via syringe in one portion. The reaction was stirred for 8 h at room temperature. Further stirring did not effect the Z/E ratio or yield of II as calculated by ^{19}F NMR spectroscopy relative to the internal standard benzotrifluoride. The ambient temperature hydrolysis of II was accomplished by the slow addition of 36 mL of 10% NaOH to the reaction mixture followed by stirring at room temperature for 18 h. (^{19}F NMR analysis of the reaction mixture revealed a Z/E ratio of 13/87, which is consistent with that of the isolated product.) The resultant organic layer was acidified and then steam distilled. The distillate was extracted with methylene chloride (2 × 25 mL), followed by washing with 40% sodium bisulfite (2 × 25 mL) and water (2 × 25 mL), and the organic portion dried with anhydrous magnesium sulfate. After solvent removal by distillation, the resulting oil was distilled, yielding 1.8 g (61% yield based on aldehyde, Z/E = 13/87) of 1-fluoro-2-phenylethene; bp[13] 71–74 °C (65 mmHg).

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Nucleophilic Displacement vs. Proton Transfer: The System OH⁻·(H₂O)₀,₁,₂ + CH₃Cl in the Relative Energy Range 0.03–5 eV

Michael Henchman,*[1] Peter M. Hierl,[2] and John F. Paulson

Air Force Geophysics Laboratory (AFSC)
Hanscom AFB, Massachusetts 01731
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When a Lewis base X^- reacts with a methyl halide CH_3Y, it may act as a Brønsted base or as a nucleophile

$$X^- + CH_3Y \rightarrow HX + CH_2Y^-$$
$$\rightarrow CH_3X + Y^-·$$
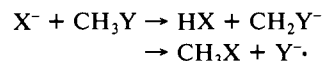
At thermal energies this competition is dominated by proton transfer when proton transfer is exothermic— and by nucleophilic displacement only when proton transfer is endothermic.[3] Here we show how proton transfer can still dominate when it is endothermic *provided there is sufficient energy available to drive it*, and we show further how this effect is suppressed by hydrating the base with one or two water molecules.

We have studied the system $OH^-·(H_2O)_{0,1,2} + CH_3Cl$ in the relative energy range 0.2–5 eV, using the AFGL tandem mass spectrometer and techniques previously described.[4,5] For OH^- + CH_3Cl nucleophilic displacement alone is exothermic, and it alone is observed at 300 K.[5-7] However, with increasing trans-

(1) AFSC-URRP Visiting Professor, 1984–1985. Permanent address: Department of Chemistry, Brandeis University, Waltham, MA 02254.
(2) USAF-SCEEE Summer Faculty Research Fellow, 1980. Permanent address: Department of Chemistry, University of Kansas, Lawrence, KS 66045.
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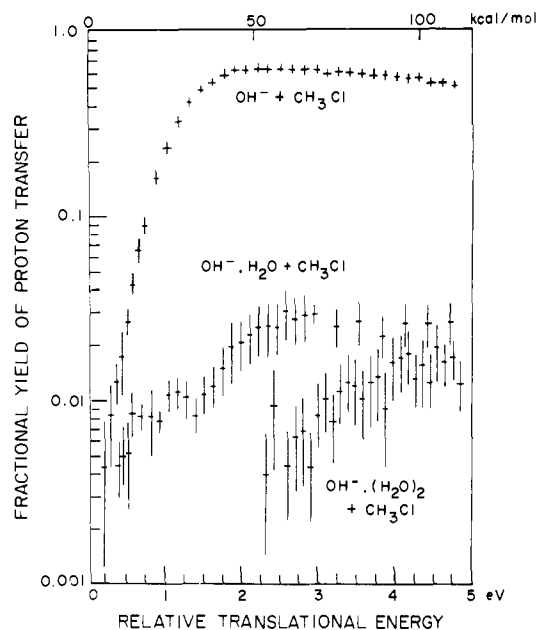(12) Foster, R. "Organic Charge-Transfer Complexes"; Academic Press: New York, 1969; pp 78–80, 303, 305.

Figure 1. Fractional abundance of proton transfer to nucleophilic displacement plus proton transfer, from experimental excitation functions (reaction cross section as a function of relative energy). For the proton-transfer channel, only CH_2Cl^- is observed, without hydrates. For the nucleophilic displacement channel, hydrated product ions comprise <10% of the product yield.[5,8]
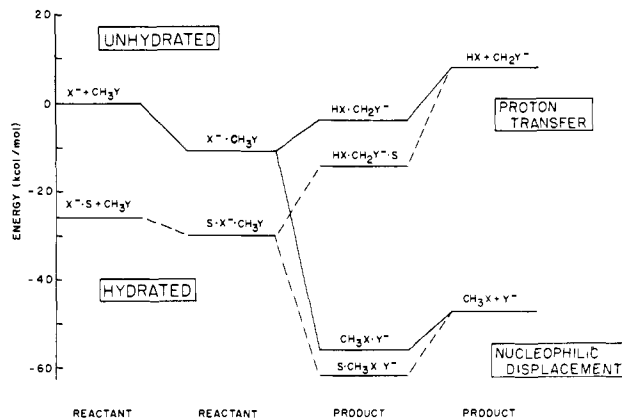

Figure 2. Energies of reactants, reaction intermediates, and products, relative to that for the unhydrated reactants, for the title reaction (X = OH, Y = Cl, S = H_2O). Energy differences shown are enthalpy differeces at 300 K (for values, see text). For clarity, the doubly hydrated system is not shown, and +S is omitted from the unhydrated system. Hydrated products, formed in zero or low yield, are not shown. Reaction pathways, linking initial and final states, are shown as straight lines without energy barriers, using dashed lines to differentiate the hydrated system.

lational energy, the endothermic proton-transfer reaction competes increasingly, becoming the major product above ~1 eV (Figure 1). In contrast, when the base OH^- is hydrated with one or two water molecules, translational energy never drives the proton transfer above a relative yield of 3% (Figure 1). Summarizing, at translational energies where both reactions can occur, the *endothermic* reaction *dominates* the highly *exothermic* reaction when the reactant ion is unhydrated but not when it is hydrated.

A simple model can account qualitatively for these findings. Both reactions—proton transfer and nucleophilic displacement—have been described successfully using potential energy profiles which feature two minima (representing the reactant adduct and the product adduct) and an energy barrier separating them.[7,9–11] Competition between proton transfer and nucleophilic displacement can then be described in terms of competitive motion along the two potential energy profiles.[12] Figure 2 shows features of these profiles, in the form of an energy-level diagram, giving estimated relative energies of reactants, intermediates, and products but omitting energy barriers.[13] This energy-level diagram can then be used to interpret the kinetic data in Figure 1.

Consider the unhydrated reactants $OH^- + CH_3Cl$. Nucleophilic displacement is exothermic ($\Delta H^\circ = -47.5$ kcal/mol)[7] and at 300 K occurs at almost every collision: proton transfer is endothermic ($\Delta H^\circ = +8$ kcal/mol)[14] and does not. Translational energy reverses this, driving *endothermic* proton transfer over *exothermic* nucleophilic displacement (Figure 1). Our explanation resolves the proton-transfer reaction into two steps (Figure 2): (1) forming the intermediate (reactants → reactant adduct) and transferring the proton within the intermediate (reactant adduct → product adduct); (2) separating the products (product adduct → products).

Step 1, $OH^- + CH_3Cl \rightarrow H_2O \cdot CH_2Cl^-$, is actually exothermic ($\Delta H^\circ = -4$ kcal/mol);[14,15] step 2, $H_2O \cdot CH_2Cl^- \rightarrow H_2O + CH_2Cl^-$, is endothermic ($\Delta H^\circ = +12$ kcal/mol).[15] The endothermic proton-transfer reaction is not endothermic to transfer the proton but to separate the products. Thus in the *competition* between nucleophilic displacement and proton transfer, proton transfer is only limited by the availability of translational energy to separate the products of the proton-transfer reaction. The competition is further influenced by the strong *positive* energy dependence of endothermic proton-transfer reactions[16] and by the *negative* energy/temperature dependence of exothermic nucleophilic displacement reactions.[8,17]

Hydrating the reactant ion[18] $OH^- \cdot H_2O + CH_3Cl$ effectively suppresses the proton-transfer reaction. Figure 2 shows how this switching of the product distribution by the hydration is reflected in a switching of the thermochemistry. Step 1 is *exothermic* for the *unhydrated* reactant ($\Delta H^\circ = -4$ kcal/mol) but becomes *endothermic* for the *hydrated* reactant ($\Delta H^\circ = +11$ kcal/mol).[19] For the hydrated reactant, the proton-transfer reaction is endothermic($\Delta H^\circ = +33$ kcal/mol)[14,18] both to separate the products *and* to drive the proton transfer within the intermediate.

Additional results support this interpretation. First, for $OH^- \cdot (H_2O)_2 + CH_3Cl$, proton transfer is again suppressed and step 1 is even more endothermic ($\Delta H^\circ = +21$ kcal/mol). Second, both results and interpretation are essentially identical for $OH^- \cdot (H_2O)_{0,1} + CH_3Br$.[8] Third, isotope exchange $OD^- + CH_3Cl \rightarrow OH^- + CH_2DCl$ competes, under drift conditions,[20,21] consistent with the mechanism proposed in Figure 2.[10b]

Neither product is hydrated—for nucleophilic displacement, as discussed previously,[5] and for proton transfer, for similar stereochemical reasons. Solvate may not transfer to the carbon

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(13) Energy barriers for proton transfer are low.[11]

(14) From the threshold measured for CH_2Cl^-.[8]

(15) Hydration energy of $CH_2Cl^- \approx 12$ kcal/mol, by analogy with that for $Cl^- = 14.4$ kcal/mol (Larson, J. W.; McMahon, T. B. *J. Am. Chem. Soc.* **1984**, *106*, 517).

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(18) Hydration energy = 25 kcal/mol (Kebarle, P. *Annu. Rev. Phys. Chem.* **1977**, *28*, 445.

(19) Second hydration energy of $CH_2Cl^- \approx 10$ kcal/mol, by analogy with that for $Cl^- = 12.7$ kcal/mol.[18]

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(21) This obviates proton transfer via stripping, which, with quenching by hydration, could provide an alternative mechanism.

because it would be weakly held, the chlorine withdrawing the negative charge from the site of the proton transfer.

$$\overset{H}{HOHO^-} + HCH_2Cl \rightarrow HOH + \overset{H}{OH{\cdot}CH_2Cl^-}$$
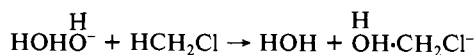
In conclusion, the reactivity of a Lewis base, in its competing roles of Brønsted base and nucleophile, has been explored as a function of translational energy. The rule at thermal energies that proton transfer prevails where it is spontaneous[3] is here extended to suprathermal energies, where proton transfer *within the intermediate* must be spontaneous for the reactants. Adding a single solvate molecule is again shown to change a reaction mechanism[22] —here, apparently, by perturbing differentially the energies of the participating species.

Registry No. CH₃Cl, 74-87-3; OH⁻, 14280-30-9.

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Mechanism of Adenylate Kinase. 1. Use of ¹⁷O NMR To Study the Binding Properties of Substrates[1]

Daniel A. Wisner, Corazon A. Steginsky, Yeun-Jund Shyy, and Ming-Daw Tsai*

Department of Chemistry, The Ohio State University Columbus, Ohio 43210

Despite the ubiquity of oxygen-containing functional groups (e.g., phosphoryl, carboxyl, and hydroxyl groups) in biological systems,^{17}O NMR has not been used to study the binding and motional properties of enzyme–substrate complexes. On the other hand, binding of small ligands to proteins has been investigated by the NMR properties of other quadrupolar nuclei such as ^{43}Ca,[2] ^{79}Br, ^{81}Br,[3] ^{35}Cl,[4] ^2H,[5] etc.

Adenylate kinase (AK) provides a good system to test the applicability of ¹⁷O NMR in enzyme–substrate interactions. The enzyme is small ($M_r \approx 21\,000$), yet consists of two distinct sites: the MgATP site binds ADP, ATP, MgADP, and MgATP, whereas the AMP site binds AMP and ADP.[6] The dissociation constants are in the order of 10^{-4}–10^{-5} M.[6b,c]

The ¹⁷O line width (ΔO) in the extreme narrowing limit ($\omega^2 \tau_c^2 \ll 1$) can be expressed by[7]

$$\Delta O = \frac{1}{\pi T_2} = \frac{1}{\pi T_1} = \frac{12\pi}{125}\left(1 + \frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{h}\right)^2 \tau_c \quad (1)$$

(1) This work was supported by research Grant GM 29041 from NIH. M.-D.T. is an Alfred P. Sloan Fellow, 1983–1985. Abbreviations: ADP, adenosine 5'-diphosphate; AK, adenylate kinase; AMP, adenosine 5'-monophosphate; ATP, adenosine 5'-triphosphate; EDTA, ethylenediaminetetraacetate; GTP, guanosine 5'-triphosphate; Hepes, N-(2-hydroxyethyl)-piperazine-N-2-ethanesulfonic acid; PPP, triphosphate; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time.

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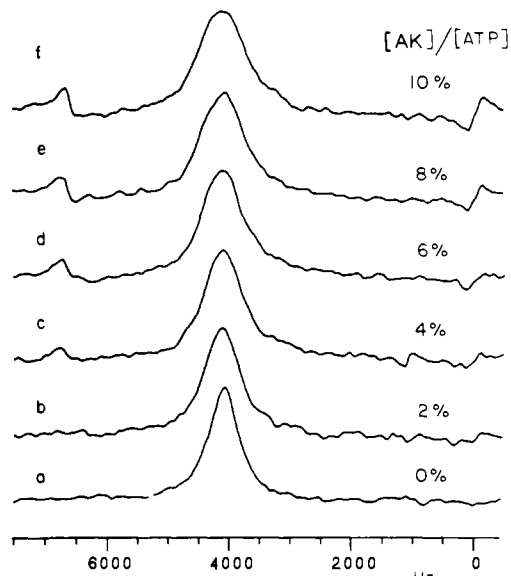
Figure 1. ¹⁷O NMR spectra (40.7 MHz) of [β-¹⁷O₂]ATP in the presence of various concentrations of AK. Sample conditions: (a) 7.0 μmol of [β-¹⁷O₂]ATP in 0.20 mL of 100 mM Hepes buffer, pH 7.9, containing 14 μmol of EDTA; (b–f) addition of 2.94 mg of AK (0.14 μmol) in 0.132 mL of 100 mM Hepes buffer (pH 7.9). ¹⁷O-depleted water (10% ¹⁷O relative to natural abundance) was used in all cases. Spectral parameters: spectral width 25000 Hz, acquisition time 41 ms, acquisition delay 20 ms, receiver gate 40 μs. The T_1 inversion–recovery program was used to suppress the solvent signal (180° = 51 μs, 90° = 25.5, μs, recovery time 4.5 ms). Temperature was 20 °C, line broadening 100 Hz, no. of transients 12000–25000. The instrument and probe have been described elsewhere.[19]
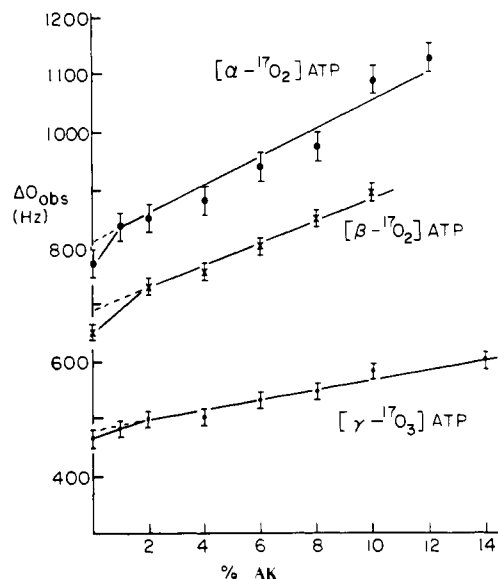

Figure 2. Dependence of ¹⁷O NMR line widths, ΔO, on the ratio $P = [AK]/[nucleotide]$. The conditions and spectral parameters are similar to Figure 1. The ¹⁷O-labeled nucleotides were available from our previous work,[19] with all ¹⁷O label at nonbridging positions of the phosphate groups. The ΔO values have not been corrected for exponential multiplication (100 Hz) and field inhomogeneity (20 Hz).

where ω is the angular frequency of ¹⁷O, τ_c is the rotational correlation time, and η and e^2qQ/h are the asymmetry parameter and the quadrupolar coupling constant, respectively, of the ¹⁷O nucleus. When a small percentage (P) of an ¹⁷O-labeled nucleotide is bound to an enzyme, the observed line width is given by[8-10]

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