cyclization products are formed in large quantitites in these adamantyl esters whether α-fluorine are present or absent.

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Models for NADH Dependent Enzymes. Solvent Effects in Dihydronicotinamide Reductions

Sir:

As part of a study to examine the hypothesis that some of the rate acceleration caused by an enzyme may be imitated by different solvent effects, we have examined the rates of reduction of trifluoroacetophenone (**2**)[1] by propyldihydronicotinamide (**1**) in various solvents and solvent mixtures as a model for the reduction of a carbonyl by an NADH dependent enzyme. Although it has been noted[2] that these kinds of reactions are sensitive to the polarity of the medium, in this communication we wish to report the dramatic effect of the protic nature of the solvent on the rate of reduction.

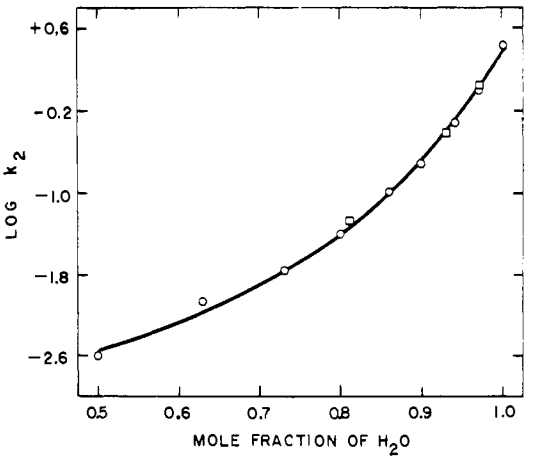
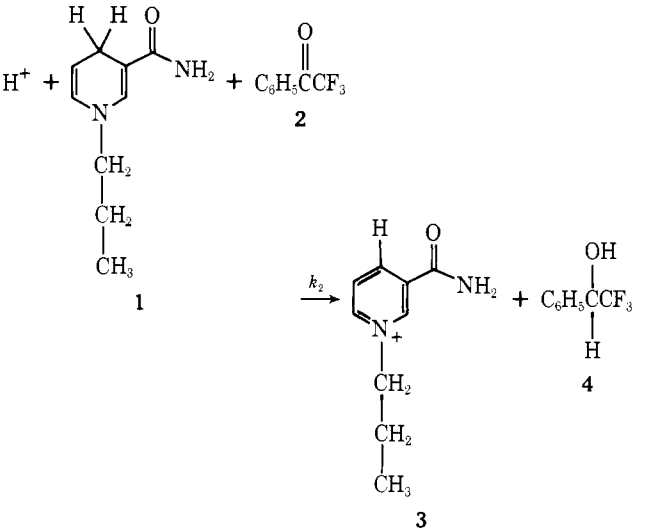


Figure 1. Dependence of log k_2 on the mole fraction of water present during the reduction of trifluoroacetophenone with propyldihydronicotinamide at 50.0 ± 0.1° in: (a) water–dimethyl sulfoxide mixture containing 0.01 M sodium carbonate–sodium bicarbonate (pH 9.9) buffer (○); (b) water–isopropyl alcohol mixtures containing 0.001 M solution dihydrogen phosphate–sodium hydrogen phosphate (pH 7.2) buffer (□).



The reductions were studied in the appropriate buffered solvent or solvent mixture maintained at 50.0 ± 0.1°. In the presence of an excess of trifluoroacetophenone, the disappearance of the dihydronicotinamide (**1**) monitored spectrophotometrically at 355 mμ follows first-order kinetics.[1a] The variation of the pseudo-first-order rate constant, k_{obsd}, with increasing concentration of trifluoroacetophenone is linear and provides the second-order rate constant for the reduction of **2** through the following equation:

$$k_{obsd} = k_{dec} + k_2[\mathbf{2}] \qquad (1)$$

All rate constants were evaluated by computer via the method of least squares. Product analyses were carried out by high pressure liquid chromatography.[3]

The reduction of **2** in several solvent mixtures is independent of pH over the range 7.2–9.9. Moreover, the rate of reduction of **2** at 50.0° in a solution consisting of 25% (v/v) isopropyl alcohol–water (pH 7.2) is independent of the ionic strength. No significant change occurs in the second-order rate constant when the ionic strength is varied from 0.002 to 0.5 by the addition of $LiClO_4$.

The very slow reduction of **2** in dimethyl sulfoxide or acetonitrile occurs with a second-order rate constant of approximately 6×10^{-4} M^{-1} min^{-1}. The addition of small quantities

Table I. Second-Order Rate Constants (k_2)[a] and Activation Parameters[b] for the Reduction of Trifluoroacetophenone with Propyldihydronicotinamide[c]

χ_{H_2O}	k_2 (min^{-1})	$\Delta G^{\ddagger}$ (kcal mol^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (cal mol^{-1} deg^{-1})
1.00	2.82 ± 0.03	20.9 ± 0.01	13.4 ± 0.1	−23 ± 1
0.97	0.99 ± 0.02	21.6 ± 0.01	14.8 ± 0.1	−21 ± 1
0.94	0.48 ± 0.01	22.1 ± 0.01	13.9 ± 0.5	−25 ± 1
0.90	0.200 ± 0.003	22.6 ± 0.01	13.4 ± 0.4	−29 ± 1
0.86	0.108 ± 0.003	23.0 ± 0.02	17.5 ± 1.6	−17 ± 1

[a] Determined by the best fit to eq 1 by the method of least squares. [b] Calculated from at least four values of k_2 at different temperatures in the range 40.0–70.0°. [c] At various mole fractions of water (χ_{H_2O}) in water–dimethyl sulfoxide mixtures containing 0.01 M sodium carbonate–sodium bicarbonate (pH 9.9) buffer (50.0 ± 0.1°).

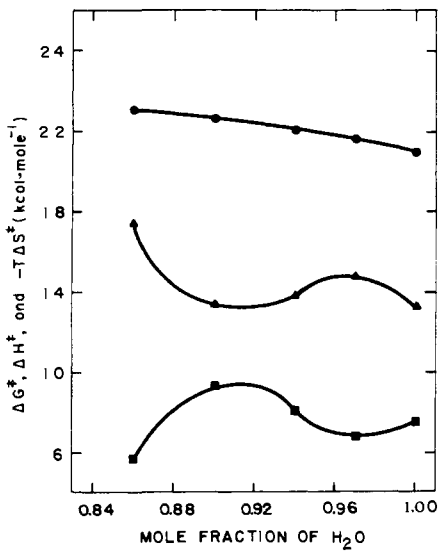


Figure 2. Variation of $\Delta G^{\ddagger}$ (●), $\Delta H^{\ddagger}$ (▲), and $-T\Delta S^{\ddagger}$ (■) for the reduction of trifluoroacetophenone by propyldihydronicotinamide at 50.0 ± 0.1° in various dimethyl sulfoxide–water mixtures containing 0.01 M sodium carbonate–sodium bicarbonate (pH 9.9) buffer.

of water to the reaction mixture drastically increases the rate of reduction of **2**. Changing the solvent from dimethyl sulfoxide to water increased the second-order rate constant from 6×10^{-4} to 2.82 ± 0.03 M^{-1} min^{-1}, a factor of approximately 5000. A steady increase in value of the second-order rate constant is observed as the mole fraction of water in water–dimethyl sulfoxide mixtures is increased (Figure 1).

To test the hypothesis that the rate acceleration was caused by the protic nature of the medium as reflected in the mole fraction of water, the variation of the second-order rate constant in water–isopropyl alcohol mixtures of intrinsically lower dielectric constant was also measured (Figure 1). These experiments indicate that the variations of the second-order rate constants as a function of the mole fraction of water in water–dimethyl sulfoxide or water–isopropyl alcohol mixtures are experimentally indistinguishable. Because the polarity, as measured by the dielectric constant of the solvent, decreases more rapidly in water–isopropyl alcohol mixtures than water–dimethyl sulfoxide mixtures as the mole fraction of water is decreased, the results suggest that the major solvent effect on the rate, in the range studied, is the availability of a polar-protic solvent.

These results can be interpreted on the basis of the work of Parker and others.[4] The observed rate enhancement may be partially attributed to the increase in the chemical potential of the reactants that results from the decreased solubility of the hydrophobic reactants as the mole fraction of water is increased. The majority of the rate enhancement is probably due to the more favorable solvation of the anionic portion of the dipolar transition state in polar-protic solvents than polar-aprotic solvents.[5] This is supported by the observation[6] that the chemical potential of OH^- ion is dramatically decreased when water is added to a solution of OH^- ion in dimethyl sulfoxide. Hence, the introduction of the polar-protic solvent (water) during the reduction provides a means of stabilizing the developing negative charge on the oxygen in the transition state through hydrogen bonding. This view is further supported by the observed importance of intramolecular hydrogen bonding in the reduction of substituted thiobenzophenone,[2c] 3-hydroxypyridine-4-carboxaldehydes,[2b] and salicylaldehyde compounds.[7]

In an attempt to dissect the nature of the rate enhancement, the activation parameters of the reduction were measured as a function of the solvent composition (Table I). These results support the notion that the action of an added component in a solvent mixture is more than a simple dilution of the solvent or a modification of its dielectric properties (Figure 2). The smooth decrease in the free energy of activation with the increasing mole fraction of water is a result of irregular but compensating fluctuations in the enthalpy and entropy of activation.

These studies demonstrate the importance of the protic nature of the solvent during the reduction. Moreover, preliminary experiments indicate that the reduction of trifluoroacetophenone in acetonitrile displays general-acid catalysis. The relevance of these studies to the enzymatic mechanism is supported by recent x-ray crystallographic studies of an abortive ternary complex of the dogfish NAD^+-dependent lactate dehydrogenase which revealed the location, with respect to the substrate, of the essential histidine 195 in the active site of the enzyme.[8] Kinetic studies[9] indicate that the binding of pyruvate to the enzyme–NADH complex compulsorily protonates his-195. This and other observations[9] are consistent with the formation of a hydrogen bond between the pyruvate and his-195 and with his-195 acting as a general acid during the reduction process.

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Chemical Probes of Nitrogenase. 1. Cyclopropene. Nitrogenase-Catalyzed Reduction to Propene and Cyclopropane[1]

Sir:

One of the more remarkable properties of nitrogenase consists in its ability to catalyze the reduction of diverse small unsaturated molecules besides N_2, the biological substrate.[2] Prominent among these "adventitious"[3] substrates is C_2H_2, which the enzyme readily reduces to C_2H_4 (but not to C_2H_6).[4] In contrast to C_2H_2, C_2H_4 is completely unreactive to nitrogenase;[5] the origin of this selectivity, an important clue towards the understanding of nitrogenase function, is unknown primarily because an appropriate investigative tool has not been available to enzymologists. It appeared to us reasonable to

$$HC \equiv CH \xrightarrow[ATP,\,2e^-]{N_2ase} H_2C = CH_2$$

$$H_2C = CH_2 \xrightarrow[ATP,\,2e^-]{N_2ase} \text{No reaction}$$

surmise that *a molecule possessing electronic properties intermediate between those of C_2H_2 and C_2H_4 might display partial reactivity with nitrogenase,* and therefore could be of use as a chemical probe to elucidate the molecular basis for such phenomena as C_2H_2 vs. C_2H_4 selectivity and other interesting aspects of nitrogenase catalysis. *Cyclopropene* as a candidate molecule of this type[6] combines a number of attractive features: (1) it clearly satisfies our fundamental criterion; i.e., it is recognized to possess properties in between those regarded as typical for unstrained alkenes and alkynes;[7] (2) it has the most compact steric profile of the three C_3H_4 isomers; and (3) its reduction conceivably could lead to any of several stable products—cyclopropane or propene via a two-electron reduction, propane via a four-electron reduction—which themselves have little or no tendency to interact further with nitrogenase.[8] In this communication we report evidence that nitrogenase prepared from *Azotobacter vinelandii* OP catalyzes the formation of *both propene* and *cyclopropane*

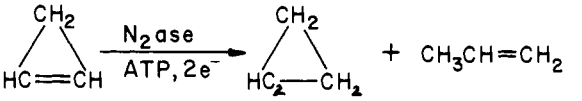

Table I. Nitrogenase-Catalyzed Reduction of Cyclopropene

Expt No.	Assay mixture	Pinit, cyclopropene (atm)[a]	Reaction time (min)	Product formed (nmol)[b] Propene	Cyclopropane	GC column
1	Complete[c]	0.025	10	452	—	*d*
			30	1.26×10^3	—	*d*
			100	1.30×10^3	6×10^2	*e*
2	None	0.02	10	<5[f]	—	*d*
	ATP, DT[g]		10	<5[f]	—	*d*
	Complete[c]		10	347	—	*d*
			57	$\sim 7 \times 10^2$	$\sim 3 \times 10^2$	*h*
			93	7.3×10^2	3.4×10^2	*e*
3	None	0.005	20	$<1 \times 10$[f]	<2[f]	*d, h*
	DT[i]		20	$<2 \times 10$[f]	<2[f]	*h*
	DT, N_2ase[j]		20	$<2 \times 10$[f]	<2[f]	*h*
	Complete[j]		20	155	79	*h*
7	None	0.04	30	<2[f]	<10[f]	*d, k*
	ATP, N_2ase[l]		30	26[f]	<10[f]	*d, k*
	Complete[m]		21.5	367	—	*d*
			105	790	375	*k*
8	None	0.03–0.015	40	<2[f]	<2[f]	*k*
	ATP, DT[n]		13	6[f]	<2[f]	*k*
	complete[o]		13	212	109	*k*
			29	466	262	*k*
10	None	0.035–0.02	20	<8[f]	<5[f]	*k*
	DT, N_2ase[p]		11	<9[f]	<8[f]	*k*
	Complete[q]		10	380	198	*k*

[a] Initial partial pressures (balance, Ar to 1 atm), determined manometrically or estimated by GC. [b] All experiments conducted at 30°. C_2H_6 used as internal standard in expt. 7, 8, and 10. Apparent activities in some experiments limited by agitation rate. [c] 4.9 mg CP-S N_2ase protein in 2 ml of assay mixture as described in text. [d] Porapak N, 2 ft × 3/16 in. [e] Porapak N, 10 ft × 1/4 in. [f] Background value. [g] 0.55 ml of H_2O, 0.80 ml of ATP-generator (ATP (12.5 μM), $MgCl_2$ (12.5 μM), CP (62.5 μM), CPK (16 units), HEPES (62.5 μM, pH 7.3)), and 0.5 ml of 0.08 M DT. [h] 4 ft × 3/16 in. $AgNO_3$/ethylene glycol/firebrick, 1.5 in. × 3/16 in. Porapak N. [i] 0.15 ml of H_2O, 0.1 ml of 0.25 M HEPES/0.05 M $MgCl_2$ (pH 7), 0.25 ml of 0.08 M DT. [j] As in *i*, plus 3.3 mg of CP-S N_2ase protein in 0.1 ml of 0.01 M sodium phosphate buffer (pH 7). Complete mixture additionally contained 0.4 ml of ATP-generator. [k] 14 ft × 3/16 in $AgNO_3$/glycerol/firebrick, 2 in. × 3/16 in. Porapak N. [l] 0.6 ml of H_2O, 0.8 ml of ATP-generator (note *g*) and 3.65 mg of CP-S N_2ase protein in 0.1 ml of 0.01 M sodium phosphate buffer (pH 7). [m] As in *l*, plus 0.50 ml of 0.08 M DT. [n] 0.8 ml of ATP-generator (note *g*), 0.65 ml of H_2O, and 0.5 ml of 0.08 M DT. [o] As in *n*, plus 1.84 mg of CP-S N_2ase protein in 0.05 ml of 0.01 M sodium phosphate buffer (pH 7). [p] 0.45 ml of 0.25 M HEPES/0.05 M $MgCl_2$ (pH 7), 0.5 ml of 0.08 M DT and 9.18 mg of CP-S N_2ase protein in 0.25 ml of 0.01 M sodium phosphate buffer, pH 7. [q] As in *p*, plus 0.8 ml of ATP-generator (note *g*).