Model Dehydrogenase Reactions. Direct Observation of a Charge-Transfer Complex in a Dihydronicotinamide Reduction

Sir:

The analyses of isotope effects in nonenzymic dihydronicotinamide reductions of *N*-methylacridinium ion, the zinc complex of 1,10-phenanthroline-2-carboxaldehyde, and trifluoroacetophenone have suggested that the formation of at least one noncovalent intermediate is partially rate limiting during the course of these reactions.[1-4] We have previously reasoned that the simplest scheme (eq 1) involving the formation of a noncovalent complex, X, from the dihydronicotinamide, RH_2, and oxidizing agent, A, is inadequate.

$$RH_2 + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X \xrightarrow{2k_H} RH + AH \qquad (1)$$

It requires that the rate of formation of X be restricted to the approximate value of the overall reaction and therefore cannot approach the diffusion-controlled limits characteristic of such species. If an additional intermediate forms subsequent to the initial aromatic complex,

$$RH_2 + A \rightleftharpoons Y_1 \rightleftharpoons \dot{Y}_2 \rightarrow RH + AH \qquad (2)$$

the above kinetic dilemma may be resolved and the unusual isotope effects can still be rationalized. In the present communication, we wish to report the direct spectroscopic observation of a kinetically competent aromatic complex in the reduction of *N*-methylacridinium ion by *N*-benzyldihydronicotinamide. The kinetic isotope effects observed with the three possible isotopic forms of *N*-benzyldihydronicotinamide suggest that this aromatic complex is Y_1 of eq 2 and support the inclusion of the additional intermediate species, Y_2.

The mixing of 0.2 M *N*-benzyl-1,4-dihydronicotinamide in anhydrous methanol with an equimolar solution of *N*-methylacridinium chloride in a stopped-flow spectrophotometer results in the appearance of an intense dark color within the 2-ms dead time of the instrument. This absorption then fades with a rate consistent with the overall rate of the net reaction. The absorption spectrum obtained by extrapolating the absorbance of the reaction mixture to zero time at a series of wavelengths (Figure 1) satisfies the well-known criteria for charge-transfer complexation in solution[5] and supports the formation of such a complex between the reactants upon mixing. The rapid rate of formation of this absorption spectrum is comparable with that observed for other charge-transfer complexes.[6] Previous studies have indicated that dihydronicotinamides can form charge-transfer complexes with maleic anhydride and flavins.[7-10]

The rate of disappearance of the charge-transfer complex was measured in methanol as a function of the concentration of dihydronicotinamide at a constant concentration of *N*-methylacridinium ion (10^{-3} M). Thedihydronicotinamide was present in excess and the pseudo-first-order rate constants at each dihydronicotinamide concentration were determined from the rate of decrease of the "instantaneous" absorption at 550 nm. Plots of the reciprocal of the observed first-order rate constants at each dihydronicotinamide concentration vs. the reciprocal of the dihydronicotinamide concentration were linear (Figure 2). The extrapolated first-order rate constant at an infinite concentration of dihydronicotinamide is 55.5 s^{-1} and the dihydronicotinamide concentration at which half the maximal rate is obtained is 0.32 M. The ratio of the first-order rate constant to this concentration or its equivalent, the inverse of the slope of the double reciprocal plot, is 173 M^{-1} s^{-1}. This is identical, within experimental error, with the second-order rate constant measured from the rate of disappearance of the individual components at concentrations where the reaction exhibits strict second-order kinetics. The important conclusion from these observations is that the charge-transfer complex is kinetically competent and therefore a possible intermediate in the overall reaction.

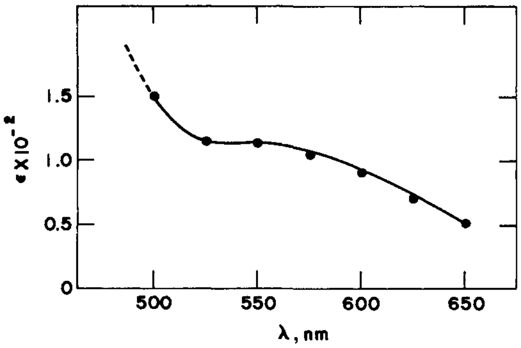

Figure 1. The absorption spectrum of the charge-transfer complex [RH_2,A] in anhydrous methanol. The points shown were determined by monitoring the rate of decomposition of the complex at different wavelengths with a stopped-flow apparatus and extrapolating the optical densities to zero time and fully saturated conditions (see Figure 2).

The demonstration of kinetic competence of the complex is not sufficient to warrant the conclusion that it lies on the main reaction pathway. It is possible that it exists in rapid equilibrium with the reactants, as indicated in eq 3. Although this kinetic ambiguity cannot be definitively excluded, kinetic

$$\begin{array}{l} RH_2 + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X \xrightarrow{2k_H} RH + AH \\ \quad \updownarrows \\ \quad Z \end{array} \qquad (3)$$

isotope studies with monodeutero and dideutero *N*-benzyldihydronicotinamide-*4*-d_1 and -*4*-d_2 argue against it.

The kinetic parameters obtained from double reciprocal plots using the monodeutero and dideutero dihydronicoti-

Table I. Isotope Effects on the Rates of Reduction of *N*-Methylacridinium Ion by Dihydronicotinamides and the Corresponding Product Isotope Partitioning

Decomposition of the charge-transfer complex				Product partitioning
Complex	$k^{25\,°C}$,[a] s^{-1}	K_D,[b] M	$\frac{1}{(1/k)/(1/[RH_2])}$,[b] l. mol^{-1} s^{-1}	$\frac{N\text{-methylacridan}}{N\text{-methylacridan-2-}d_1}$
[RH_2, A]	55.5	0.32	1.73×10^2	4.0 ± 0.4[c]
[RH_2-*4*-d_1, A]	47.6	0.32	1.48×10^2	
[RH_2-*4*-d_2, A]	40.0	0.32	1.25×10^2	

[a] In anhydrous methanol (distilled from *N*-methylacridinium chloride). [b] Determined from data presented in Figure 2. [c] Determined by product analysis using NMR.

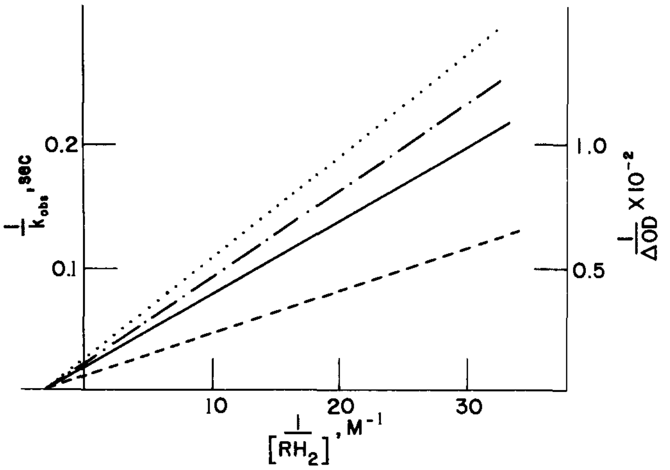

Figure 2. The double reciprocal plot of the rate of decomposition of the complex: — [RH_2,A]; - · - · - · [RH_2-*4*-d_1,A]; · · · · [RH_2-*4*-d_2,A]. The concentration of the oxidant was fixed at 1×10^{-3} M and the reaction was followed at 550 nm. The lowest curve (- - -) corresponds to the reciprocal $A_0 - A_\infty$ determined from the kinetic runs for each concentration of dihydronicotinamide used.

namide derivatives indicate that the concentration of dihydronicotinamide at which the rate is half maximal is identical with that for the dihydro form. Since this parameter is independent of the isotopic form of the dihydronicotinamide used and is the same as that obtained from plotting the initial absorbance increase as a function of dihydronicotinamide, this concentration must represent the dissociation constant of the charge-transfer complex. Of perhaps greater significance is that the pseudo-first-order rate constant extrapolated at infinite dihydronicotinamide concentration does not exhibit a substantial primary isotope effect even though a substantial isotope partitioning ratio is observed in the product *N*-methylacridan when monodeutero *N*-benzyldihydronicotinamide-*4*-d_1 is used as the reducing agent (Table I). Therefore these data obtained at saturating concentrations of dihydronicotinamide exhibit the same divergence of the kinetic isotope effect and the isotope partitioning ratio as was observed previously under strict biomolecular conditions for *N*-propyldihydronicotinamide.[1]

The most definitive statement that can be made from these data is that the charge-transfer complex observed cannot be the intermediate indicated in eq 1, as in this case a pronounced isotope effect would be expected on the extrapolated pseudo-first-order rate constant. Although the scheme summarized in eq 3 cannot be absolutely excluded, it must be indicated that if the kinetically competent charge-transfer complex is considered to be Z of that scheme, the problems of postulating the existence of a single noncovalent complex such as X, which have been discussed above, still remain.

In view of the recent suggestion of (a) the likely importance of radical ion pairs in flavin and dihydronicotinamide reductions,[11] (b) the expectation that such intermediates might form via complexes of the nature identified here, and (c) the a priori argument favoring the formation of two intermediates in any case, we consider that the scheme summarized in eq 2 most accurately represents the detailed mechanism of the dihydronicotinamide reduction of *N*-methylacridinium ion. The direct demonstration of intermediate Y_2 and the elucidation of its chemical nature should be valuable in understanding the mechanisms of chemical and enzymic dihydronicotinamide reductions.

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Nasute Termite Soldier Frontal Gland Secretions. 1. Structure of Trinervi-2*β*,3*α*,9*α*-triol 9-*O*-Acetate, a Novel Diterpene from *Trinervitermes* Soldiers

Sir:

The defensive secretions ejected from the nozzle-like frontal glands of *Trinervitermes* and *Nasutitermes* soldiers (Isoptera: Termitidae: Nasutitermitinae) have been reported[1-5] to contain polyisoprenoids of unknown structure dissolved in monoterpene hydrocarbons. These sticky secretions mechanically disable ant predators,[6] and also appear to possess some systemic toxicity to the ants.[4,7-9]

We wish to report the results of x-ray crystallographic studies carried out on one of the major constituents, "TG-2", of the soldier secretions of *Trinervitermes gratiosus* Sjostedt. It is a novel diterpene skeleton with a bridgehead double bond in an 11-membered ring.

Trinervi-2*β*,3*α*,9*α*-triol 9-*O*-acetate crystallized upon slow evaporation of an ether solution under an argon atmosphere. The crystals formed in the monoclinic space group *C*2 with $a = 15.726$ (3) Å, $b = 10.903$ (2) Å, $c = 25.749$ (5) Å, and $\beta = 106.52$ (1)°. The volume indicated that the asymmetric unit consisted of two complete molecules of $C_{22}H_{34}O_4$ for a physically reasonable calculated density of 1.14 g cm^{-1}. All unique diffraction maxima within a 2θ sphere of 114° were measured on a fully automated four-circle diffractometer using graphite monochromated Cu Kα radiation (1.5418 Å). The crystal was sealed in a Lindemann capillary. Of the 3092 reflections measured, 2297 (74%) were considered to be observed ($F_o^2 \geq 3\sigma(F_o^2)$) after correction for polarization, background, and Lorentz effects.

An initial phasing of the normalized structure factors was done with a weighted, multiple solution tangent formula approach.[10] Among the solutions with the lowest figures of merit was one displaying a chemically reasonable eight atom fragment. When this fragment was displaced 1.25Å in the *z* direction and used to calculate starting phases for tangent formula recycling, additional chemically reasonable atoms appeared which eventually were elaborated into a complete trial structure. Full-matrix least-squares refinements[11] with anisotropic temperature factors for the nonhydrogen atoms and isotropic temperature factors for the hydrogens lowered the