species.[14] The present work constitutes the first structural indications of what could be the active site in the reduced pentacoordinate state of P-450. Indeed, I contains a high-spin ($S = 2$) pentacoordinate ferrous mercaptide porphyrin species. Solutions of I in chlorobenzene present a magnetic moment of 4.8 ± 0.1 μ_B (NMR) indicating that the pentacoordinate high-spin iron(II) complex is also predominant in that medium.[6] The visible spectrum presents bands at 422 (5.13), 530 (3.87), 580 (3.73), 630 (3.60) nm in chlorobenzene. These solutions quickly pick up CO to give rise to a compound displaying a hyper-porphyrin-type spectrum with bands at 386 (4.94), 458 (4.96), 565 (3.63), and 615 (3.63) nm.[15] CO absorption can be followed in the infrared where a new absorption appears at 1920 cm^{-1}. This reaction is completely reversible and the spectrum of I is regenerated when the UV cell is purged with argon. It is noteworthy that CO uptake also occurs reversibly in the solid state (ν_{CO} 1930 cm^{-1}).

The carbonyl adduct has been crystallized as the TTP complex (II) and an X-ray analysis at 75 K has confirmed the presence of CO at the sixth coordination site. II is diamagnetic in solution. The porphyrin ring is planar. At the present stage of refinement, the average Fe–N_p bond distance is 1.993 (4) Å and a general contraction of the porphyrinato core attributable to a high-spin to low-spin transition of the iron atom takes place (0.04 Å). The displacement of the iron atom with respect to the mean plane of the four nitrogen atoms (0.02 Å) is within experimental error. The Fe–S bond distance of 2.352 (2) Å is not significantly different from that of 2.360 (2) found in I.[16] Fe–C and C–O bond lengths (1.78 (1) and 1.17 (1) Å) are not significantly different from those present in Fe(TTP)(py)(CO).[17]

Mossbauer spectral studies of I and II and X-ray studies of other iron mercaptide porphyrin complexes are presently underway.

Acknowledgments. We thank Professor J. M. Lehn (Université Louis Pasteur) for a substantial gift of the [2.2.2] cryptand.

References and Notes

(1) I. C. Gunzalus, T. C. Pederson, and S. G. Sligar, *Annu. Rev. Biochem.*, **44**, 377 (1975).
(2) D. R. Morris and L. P. Hager, *J. Biol. Chem.*, **241**, 1763 (1966).
(3) P. F. Hollenberg and L. P. Hager, *J. Biol. Chem.*, **248**, 2630 (1973).
(4) J. O. Stern and J. Peisach, *J. Biol. Chem.*, **249**, 7495 (1974).
(5) (a) J. P. Collman, T. N. Sorrell, J. H. Dawson, J. R. Trudell, E. Brunnenberg, and C. Djerassi, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 6 (1976); (b) J. P. Collman and T. N. Sorrell, *J. Am. Chem. Soc.*, **97**, 4133 (1975).
(6) (a) C. K. Chang and D. Dolphin, *J. Am. Chem. Soc.*, **97**, 5948 (1975); (b) C. K. Chang and D. Dolphin, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3338 (1976).
(7) S. C. Tang, S. Koch, G. C. Papaefthymiou, S. Foner, R. B. Frankel, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, **98**, 2414 (1976).
(8) J. P. Collman, T. N. Sorrell, K. O. Hodgson, A. K. Kulshresta, and C. E. Strouse, *J. Am. Chem. Soc.*, **99**, 5180 (1977).
(9) S. P. Cramer, J. H. Dawson, K. O. Hodgson, and L. P. Hager, *J. Am. Chem. Soc.*, **100**, 7282 (1978).
(10) (a) Preparation of compound I was carried out in C_6H_5Cl using FeTPP and [Na⊂222]SC_2H_5. It crystallizes as a bischlorobenzene solvate in space group $P2_1/c$ with a = 13.238 (4), b = 20.999 (6), c = 25.456 (7) Å; β = 94.85 (5)°; Z = 4. A total of 10 332 reflections were recorded at room temperature using a Philips PW1100 diffractometer with filtered Cu Kα radiation ($\theta/2\theta$ flying step scan); 5627 reflections with $I > 3\sigma(I)$ were included in subsequent calculations. (b) Compound II was prepared in C_6H_6 using Fe(CO)TTP and [Na⊂221]SC_2H_5 under CO atmosphere. It crystallizes as a 1.5C_6H_6 and 1[Na⊂221]SC_2H_5 solvate in space group $P\bar{1}$ with a = 21.255 (15), b = 20.696 (13), c = 11.311 (8) Å; α = 93.03 (3), β = 79.43 (4), γ = 108.21 (3)°; Z = 2. To improve diffraction, data were collected at 75 K on a Picker FACS I diffractometer equipped with a CT-38 Cryogenic Association low-temperature device using Mo Kα radiation. A total of 16 500 reflections were measured in the $\theta/2\theta$ integrating scan mode and 5520 observations with $I > 3\sigma(I)$ were retained and used in the refinement. Structure solution and refinement were performed on a PDP 11/60 computer using the Enraf-Nonius structure determination package. The present $R_1 = (\Sigma||F_o| - |F_c||)/|F_o|$ values are, respectively, 0.087 for I with anisotropic temperature factors for all nonhydrogen atoms and 0.098 for II with anisotropic temperature factors for Fe, S, and Na^+; all other atoms were given isotropic temperature factors.
(11) J. L. Hoard in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, pp 317–380.
(12) C. B. Jameson, F. S. Molinaro, J. A. Ibers, J. P. Collman, J. I. Brauman, E. Rose, and K. S. Suslick, *J. Am. Chem. Soc.*, **100**, 6769 (1978).
(13) R. T. Tsai, C. A. Yu, I. C. Gunzalus, J. Peisach, W. Blumberg, W. H. Orme-Johnson, and H. Beinert, *Proc. Natl. Acad. Sci. U.S.A.*, **66**, 1157 (1970).
(14) (a) M. Sharrock, P. D. Debrunner, J. P. Lipscomb, V. Marshall, and I. C. Gunzalus, *Biochim. Biophys. Acta*, **420**, 8 (1976); (b) P. Champion, J. D. Lipscomb, E. Münck, P. Debrunner, and I. C. Gunzalus, *Biochemistry*, **14**, 4151; 4159 (1975).
(15) Similar spectra were obtained for the carbonyl adduct with other porphyrins, e.g.: OEP in benzene, 370, 450, and 544; PPIXDME, 380, 460, and 555 (378, 455, and 555 in toluene).
(16) The asymmetric unit of II contains one cocrystallized [Na⊂221]SC_2H_5 in addition to that required by stoichiometry. The thiolate anion is free and does not interact with the porphyrinato anion core in any way. Its closest neighbors are methylene groups and oxygen and nitrogen atoms of the [Na⊂221] cations located at distances ranging from 3.7 to 4.5 Å. As a result of the presence of a second [Na⊂221], the crystal packing thus consists of alternating layers of cryptate cations and porphyrinato anions.
(17) S. M. Peng and J. A. Ibers, *J. Am. Chem. Soc.*, **98**, 8032 (1976).
(18) Laboratoire de Cristallochimie associé au C.N.R.S. (ERA 08).

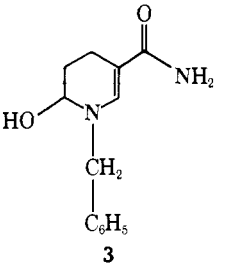
Christine Caron, André Mitschler, Georges Rivière
Louis Ricard, Michel Schappacher, Raymond Weiss*
Laboratoire de Cristallochimie,[18] Institute Le Bel
Université Louis Pasteur, 4 Rue Blaise Pascal
67070 Strasbourg, Cedex, France
Received July 16, 1979

Models for NADH Coenzymes. Evidence for an Electron-Transfer Mechanism Yielding a Radical-Cation Intermediate in the *N*-Benzyldihydronicotinamide–*N*-Benzylnicotinamide Salt Transhydrogenation Reaction

Sir:

The study of the oxidation–reduction reactions of models for nicotinamide coenzymes has provided important information about the mechanism of such processes.[1] We have been investigating the redox mechanism of the *N*-benzyl-1,4-dihydronicotinamide (**1**)–*N*-benzylnicotinamide salt (**2**) transhydrogenation reaction as a model reaction for an NADH dependent redox process[2] (Scheme I). In this communication, we report two major findings: one, the presence of the nicotinamide salt catalyzes the hydration of the dihydronicotinamide, and two, the presence of the nicotinamide salt catalyzes the exchange of the C-4 hydrogen of the dihydronicotinamide with the hydrogen from water. We believe that these observations provide evidence for an electron-transfer mechanism with an intermediate radical–radical-cation pair during the course of transhydrogenation.

An examination of a reaction mixture consisting of 0.05 M *N*-benzyldihydronicotinamide[3] (**1**) and 0.05 M *N*-benzylnicotinamide chloride (**2**) in 0.1 M (pH 8.6) aqueous phosphate by high pressure liquid chromatography (HPLC) revealed the formation of a new product during the course of transhydrogenation at 40 °C. This product was identified as the primary hydration product of *N*-benzyldihydronicotinamide, **3**, by a



3

comparison of spectral and chromatographic properties with those of an authentic sample prepared by the acid-catalyzed hydration of *N*-benzyldihydronicotinamide.[4] The formation of **3** is catalyzed by the presence of 0.1 M phosphate (pH 8.6). If the transhydrogenation is carried out in 0.1 M carbonate or

0002-7863/79/1501-7402$01.00/0

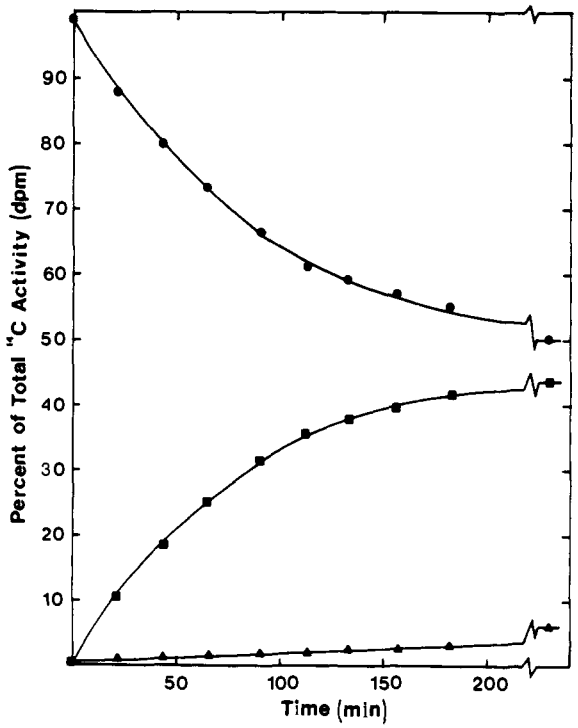
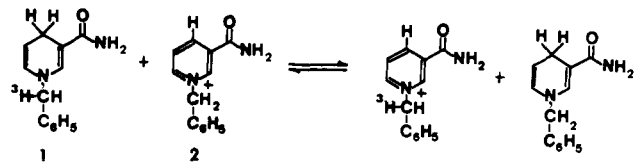


Figure 1. Time course of the *N*-benzyldihydronicotinamide (■)-*N*-benzylnicotinamide salt (●) transhydrogenation reaction and the formation of the primary hydration product of the dihydronicotinamide (▲). The reaction mixture consisted of 0.046 M *N*-benzyldihydronicotinamide and 0.043 M [7-^{14}C]-*N*-benzylnicotinamide chloride in an acetonitrile–0.1 M aqueous phosphate (pH 8.6) solvent mixture (1:3, v/v) maintained at 40.0 ± 0.1 °C. Infinity points were taken at 420 min.

Scheme I



borate buffers (pH 8.6–9.0), the formation of **3** is *not* observed during two half-lives of transhydrogenation. The course of transhydrogenation and hydration product formation was monitored by using a radiochemically labeled dihydronicotinamide or nicotinamide salt and observing the change in the specific activity of the reaction components as a function of time. The reaction components were separated by HPLC and assayed for radioactivity by liquid scintillation counting after correcting for quenching. Since the sum of the radioactivity over all components remained constant during the reaction and was equal to the total radioactivity injected into the chromatograph, the progress of the reaction was evaluated by observing the percent of the total radioactivity of each component as a function of time. A typical run is shown in Figure 1.

The observation that the rate of formation of the hydration product, **3**, is catalyzed by the nicotinamide salt, **2**, provides an important clue to the mechanism of hydration. An HPLC study of a series of reaction mixtures consisting of 0.04 M [*benzyl*-^{3}H]-*N*-dihydronicotinamide and various concentrations of nicotinamide salt (0–0.05 M) demonstrated that the initial rate of ^{3}H appearance in the hydration product was linearly dependent on the concentration of nicotinamide salt (Figure 2). Control experiments with added sodium chloride (0.5 M) demonstrated that this hydration reaction is insensitive to increases in ionic strength. The slope of $(3.3 \pm 0.2) \times 10^{-2}$ M^{-1} min^{-1} represents the second-order rate constant of nicotinamide-catalyzed hydration at the indicated conditions.

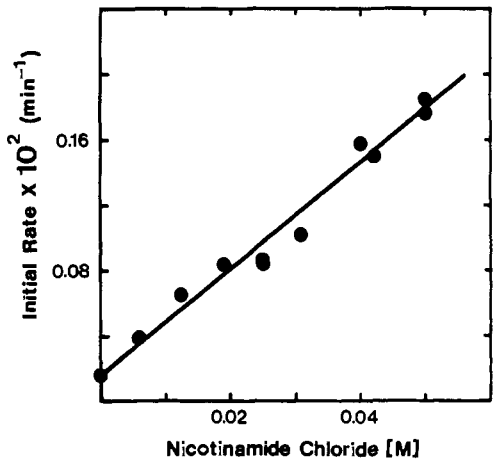


Figure 2. Variation of the initial rate of ^{3}H appearance in the primary hydration product, **3**, measured by HPLC as a function of the concentration of added nicotinamide salt. Reactions were carried out with 0.04 M [*benzyl*-^{3}H]-*N*-dihydronicotinamide in an acetonitrile–0.1 M aqueous phosphate (pH 8.6) solvent mixture (1:3, v/v) maintained at 40.0 ± 0.1 °C.

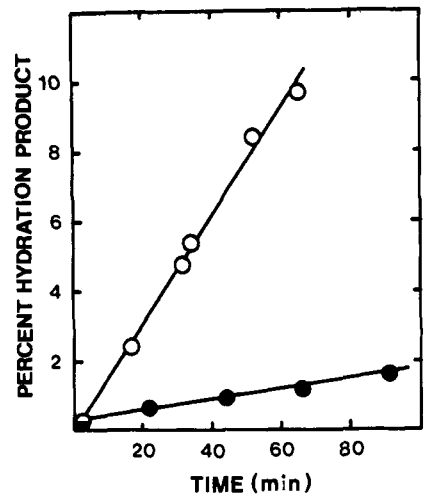


Figure 3. Time course of the appearance of ^{3}H activity (○) and ^{14}C activity (●) in the primary hydration product, **3**, expressed as the percent of the total radioactivity in the reaction mixture. Reactions containing 0.04 M [*benzyl*-^{3}H]-*N*-dihydronicotinamide and 0.04 M [7-^{14}C]-*N*-benzylnicotinamide chloride were carried out in an acetonitrile–0.1 M aqueous phosphate (pH 8.6) solvent mixture (1:3, v/v) at 40.0 ± 0.1 °C. The plot represents the results of several duplicate experiments.

The hydration product, **3**, originates from the dihydronicotinamide rather than from the nicotinamide salt during the course of transhydrogenation. A time course study of a reaction mixture consisting of [*benzyl*-^{3}H]-*N*-dihydronicotinamide and [7-^{14}C]-*N*-benzylnicotinamide chloride in a 0.1 M aqueous phosphate (pH 8.6) solvent mixture demonstrated that the initial rate of ^{3}H appearance in the hydration product is ten times faster than the initial rate of ^{14}C appearance in the hydration product (Figure 3). This observation indicates that in the initial stages, before much transhydrogenation has occurred, ~9 out of 10 molecules of hydration product, **3**, originated from the dihydronicotinamide. When the reaction mixture was examined over longer time periods (Figure 4) the rates of ^{3}H and ^{14}C appearance in the hydration product became equal in the later stages of the reaction as a result of the statistical distribution of ^{3}H and ^{14}C labels among the dihydronicotinamide and nicotinamide salt as a consequence of transhydrogenation.

The kinetic isotope effect resulting from ^{3}H substitution at C-4 of the dihydronicotinamide, in the nicotinamide salt catalyzed hydration, was calculated from the measured compe-

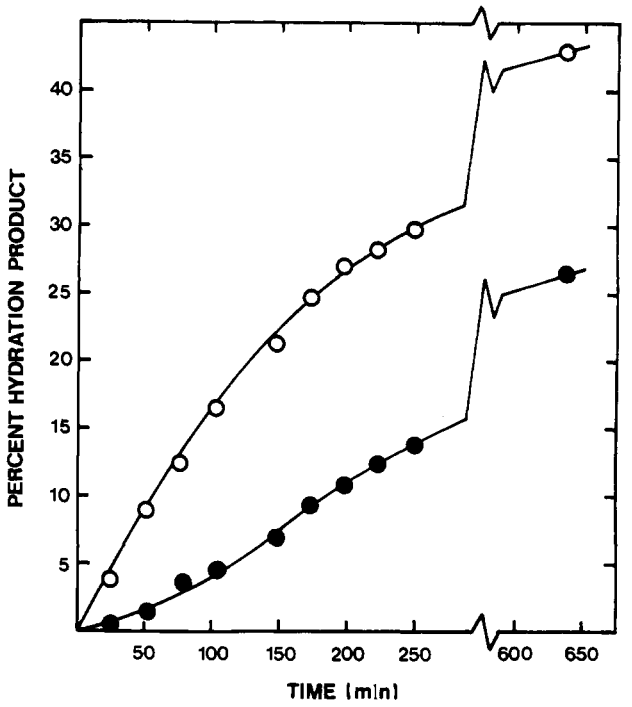


Figure 4. Extended time course of the appearance of ^3H activity (○) and ^{14}C activity (●) in the primary hydration product, **3**, expressed as the percent of the total radioactivity in the reaction mixture. A reaction containing 0.05 M [*benzyl*-^3H]-*N*-dihydronicotinamide and 0.05 M [7-^{14}C]-*N*-benzylnicotinamide chloride was carried out in an acetonitrile–0.1 M aqueous phosphate (pH 8.6) solvent mixture (1:3, v/v) maintained at 40.0 ± 0.1 °C.

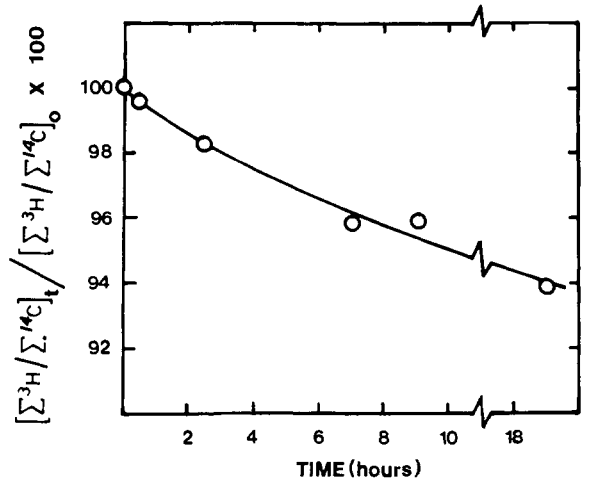


Figure 5. Time course of a transhydrogenation reaction demonstrating the loss of ^3H from the C-4 positions of the redox pair. A reaction mixture containing equimolar quantities (total concentration, 0.052 M) of [4-^3H]-*N*-benzyldihydronicotinamide, [7-^{14}C]-*N*-benzyldihydronicotinamide, [4-^3H]-*N*-benzylnicotinamide chloride, and [7-^{14}C]-*N*-benzylnicotinamide chloride was incubated in an acetonitrile–0.1 M (pH 8.8) borate buffer mixture (1:3, v/v) maintained at 70.0 ± 0.1 °C.

tition reaction between [7-^{14}C]-*N*-benzyldihydronicotinamide and [4-^3H]-*N*-benzyldihydronicotinamide to form the hydration product in the presence of 0.025 M *N*-benzylnicotinamide chloride in 0.1 M (pH 8.6) phosphate buffer. The ratio of the specific rates of the two dihydronicotinamides (kinetic ratio) was obtained from the fractional conversion of the two radiochemically labeled dihydronicotinamides into the hydration product by a procedure described previously[2] providing a kinetic ratio of $k_H/k_T = 1.03 \pm 0.02$.

In the presence of the nicotinamide salt, the C-4 hydrogen of the dihydronicotinamide undergoes exchange with the hydrogens from the solvent. This exchange can be demonstrated via two types of experiments.

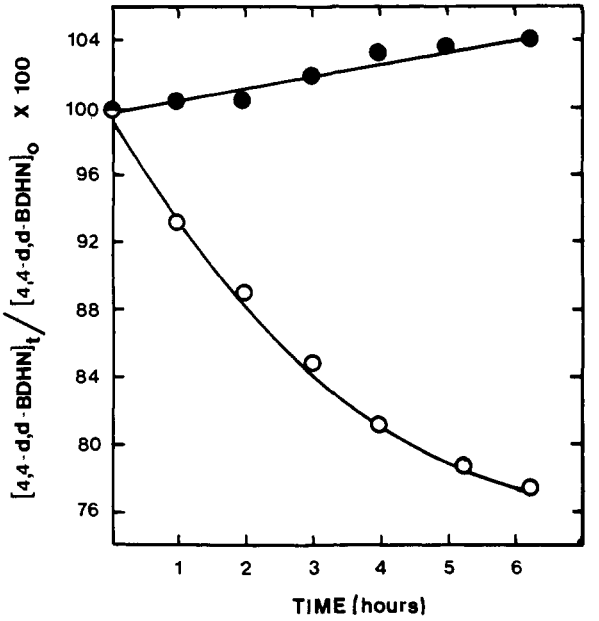


Figure 6. Time course of a transhydrogenation reaction demonstrating the loss of deuterium from the C-4 position of the dihydronicotinamide (○) catalyzed by the nicotinamide salt. A reaction mixture consisting of 0.049 M [4,4-$^2H_2$]-*N*-benzyldihydronicotinamide and 0.037 M [4-^2H]-*N*-benzylnicotinamide chloride in acetonitrile–0.1 M (pH 8.8) aqueous borate buffer mixture (1:3, v/v) is maintained at 70.0 ± 0.1 °C and analyzed by mass spectrometry. A control reaction mixture (●), where the nicotinamide salt has been left out, shows no loss of deuterium.

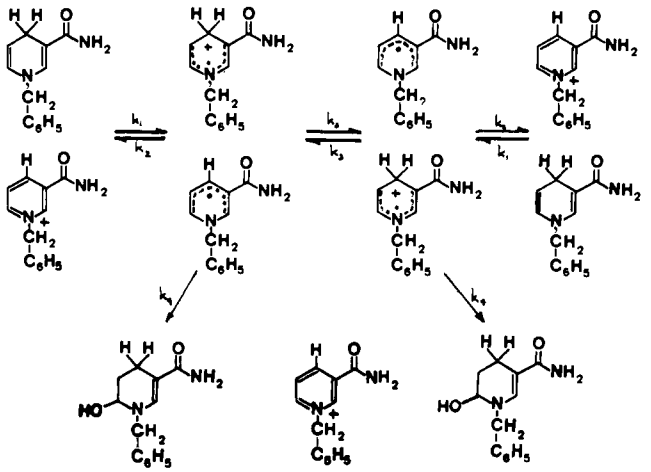
A transhydrogenation mixture consisting of equimolar quantities (total concentration, 0.052 M) of [4-^3H]-*N*-benzyldihydronicotinamide, [7-^{14}C]-*N*-benzyldihydronicotinamide, [4-^3H]-*N*-benzylnicotinamide chloride, and [7-^{14}C]-*N*-benzylnicotinamide chloride is incubated in an acetonitrile–0.1 M (pH 8.8) borate buffer mixture (1:3, v/v) at 70 °C. Under these conditions, the half-life of transhydrogenation is 12 min. Aliquots were removed at various times; the dihydronicotinamide and the nicotinamide salt were separated by HPLC, collected and analyzed for ^3H and ^{14}C activity. The percent of tritium remaining in the redox pair at any time (t) compared with that at the beginning of the reaction (0) can be readily determined from the following relationship

$$\%\ ^3H = [(\sum^3H/\sum^{14}C)_t/(\sum^3H/\sum^{14}C)_0] \times 100 \quad (1)$$

where Σ represents the sum of the radioactivity in the dihydronicotinamide and the nicotinamide salt. During the course of 45 half-lives of transhydrogenation we observe that the percent of tritium in the redox pair decreases. The time dependence of this tritium loss is shown in Figure 5. The tritium exchanged from the C-4 position of the redox pair can be located in the HPLC eluant corresponding to the "water peak". The HPLC eluant directly after the void volume of the HPLC column is rich in tritium and very low in ^{14}C as would be expected for the HPLC analysis of a tritiated water sample. The amount of tritium found in the "water peak" accounts for the tritium lost from the C-4 position of the redox pair.

The catalysis of hydrogen exchange from the C-4 position of the dihydronicotinamide by the nicotinamide salt could also be demonstrated by mass spectrometry. A transhydrogenation mixture consisting of [4,4-$^2H_2$]-*N*-benzyl-1,4-dihydronicotinamide (0.049 M) and [4-^2H]-*N*-benzylnicotinamide chloride (0.037 M) was incubated at 70 °C in the same solvent mixture as the previous experiment.[4] At various times, aliquots were removed and the dihydronicotinamide was separated by extraction into dichloromethane. The dihydronicotinamide was

Scheme II



analyzed for deuterium content by mass spectrometry using the solid probe inlet. The amount of mono- and dideuterium-labeled dihydronicotinamides was ascertained by comparing the respective 215 and 216 molecular ions of the reaction extracts with those of samples of known deuterium composition. We observe that during the course of transhydrogenation the amount of dideuterium-labeled dihydronicotinamide decreases with a corresponding increase in the monodeuterium-labeled dihydronicotinamide. The time dependence of this C-4 deuterium exchange process with the solvent is shown in Figure 6. In sharp contrast to the above result, an analogous incubation mixture where the nicotinamide salt has been left out shows no exchange (Figure 6) but rather a modest enrichment. The latter observation is probably due to a small amount of air oxidation of the dihydronicotinamide with a kinetic preference for the singly labeled compound.

In this communication we have demonstrated that the nicotinamide salt, **2**, catalyzes the hydration of the dihydronicotinamide under conditions where the acid-catalyzed mechanism is negligible. This conclusion is based on the first-order dependence of the hydration on both the dihydronicotinamide and nicotinamide salt as measured by radioactive labeling. In analogy to the known specific acid- and phosphate-catalyzed hydration of nicotinamides,[5] these observations suggest that the nicotinamide salt functions to develop positive charge at the C-6 position of the dihydronicotinamide prior to the formal addition of water across the C-5 double bond. We propose that the developed positive charge at the C-6 position of the dihydronicotinamide is the consequence of electron transfer from the dihydronicotinamide to the nicotinamide salt to form a radical–cation-radical pair.[6] We further propose that the radical–cation-radical pair lies on the reaction pathway toward transhydrogenation (Scheme II). The proposed scheme is consistent with the observed kinetic behavior of both the transhydrogenation[2] and the nicotinamide salt catalyzed hydration of the dihydronicotinamide. The observation[2] that a primary deuterium isotope effect of 6 is obtained for the transhydrogenation reaction indicates that the hydrogen-transfer step is at least partially rate determining and that the value of k_3 is smaller than k_2 in Scheme II. Hence, one would predict the absence of a primary isotope effect in the nicotinamide salt catalyzed hydration of the dihydronicotinamide and this is demonstrated by our experiments. Furthermore, the observation that the hydration product originated from the dihydronicotinamide rather than the nicotinamide salt is also in agreement with the proposed scheme.[7]

The electron-transfer mechanism outlined in Scheme II is further supported by the observed nicotinamide salt catalyzed exchange of the C-4 hydrogen of the dihydronicotinamide. Control experiments indicate that this exchange is strictly dependent on the presence of the nicotinamide salt. These observations suggest the formation of an intermediate capable of undergoing hydrogen transfer with solvent, namely the radical cation of the dihydronicotinamide.[8] Such radical cations are known to be strong acids.[9,10] Moreover, carbamidopyridinyl radicals generated via one-electron addition to pyridinium ions are known to disproportionate to pyridinium and dihydropyridine in addition to giving dimerization products.[10] Although we cannot isolate dimerization products, we see numerous decomposition products that could arrive from the breakdown of the dimer.

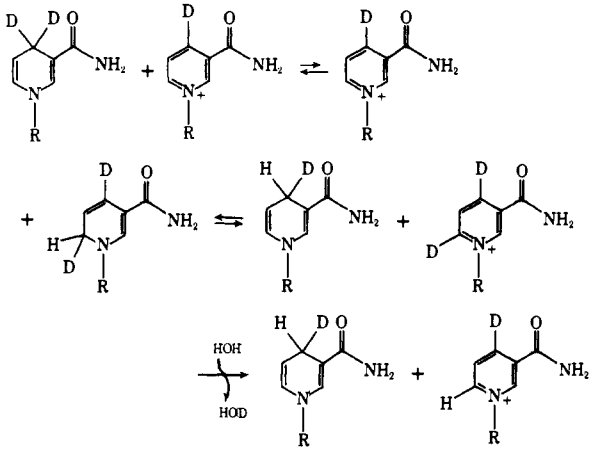
A comparison of the rate of transhydrogenation with the rate of C-4 hydrogen exchange indicates that the latter process is ~50 times slower. The very slow hydrogen exchange with solvent relative to transfer to the oxidant is consistent with the widely recognized observation that enzymic and model NADH reactions occur with specific transfer of the hydrogen from NADH to the oxidant without loss to solvent.[11] To the best of our knowledge, the transhydrogenation reaction represents the first example where this very slow loss to solvent has been measured. Our ability to measure this slow exchange rests on the unique property of the transhydrogenation reaction, namely that the process is "perpetual" owing to the symmetry between reactants and products.

In summary, the observations reported in this communication support a scheme where the electron transfer from *N*-benzyldihydronicotinamide to *N*-benzylnicotinamide salt leads to an intermediate radical–radical-cation pair which may undergo one of three further possible processes: (a) hydrogen transfer from the radical cation to the radical leading to transhydrogenation, (b) reaction of the radical cation with water leading to hydration of the dihydronicotinamide, and (c) hydrogen transfer from the radical cation to solvent leading to exchange. The existence of one-electron-transfer process in the model redox systems for NADH coenzymes suggests the possibility that similar processes occur in the enzyme-catalyzed reactions.

Acknowledgment. We acknowledge the Research Corporation and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. Furthermore, we express our appreciation to Drs. P. C. Myhre, A. K. Colter, D. M. Chipman, and L. Kurz for encouraging and helpful discussions.

References and Notes

(1) See, for instance: (a) Kurz, L. C.; Frieden, C. *Biochemistry* **1977**, *16*, 5207. (b) Colter, A. K.; Saito, G.; Sharom, F. J. *Can. J. Chem.* **1977**, *55*, 2741. (c) Hadju, J. S.; Sigman, D. S. *J. Am. Chem. Soc.* **1976**, *98*, 6060. (d) van Eikeren, P.; Grier, D. L. *Ibid.* **1976**, *98*, 4655. (e) Hadju, J. S.; Sigman, D. S. *Ibid.* **1976**, *98*, 4619.
(2) van Eikeren, P.; Grier, D. L. *J. Am. Chem. Soc.* **1977**, *99*, 8057.
(3) All dihydronicotinamides considered are 1,4 isomers.
(4) The starting dihydronicotinamide and nicotinamide salt contained 88 ± 3% deuterium at the C-4 position as determined by NMR.
(5) Johnston, C. C.; Gardner, J. L.; Suelter, C.; Metzler, D. E. *Biochemistry* **1963**, *2*, 689.
(6) The observation that dihydronicotinamides form charge-transfer complexes with the corresponding nicotinamide salts (Blankenhorn, G. *Eur. J. Biochem.* **1976**, *67*, 67) might suggest that the charge-transfer complexation is the origin of the catalysis. A strong argument against this hypothesis may be found in the report (Shinkai, S.; Ando, R.; Kunitake, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1914) that the micelle *N*-laurylnicotinamide bromide, which forms a charge-transfer complex with *N*-lauryldihydronicotinamide inhibits the specific acid-catalyzed hydration of the latter by over three orders of magniture. The possibility remains, however, that the charge-transfer complex aids in the general acid-catalyzed reaction.
(7) In an earlier report,[2] we suggested that the symmetry between reactants and products required that the energy profile of the transhydrogenation reaction be symmetric and on that basis eliminated a mechanism which involves $e^-/H\cdot$ transfer. We now realize that this assumption is questionable. Thus, although Scheme II depicts a mechanism involving the sequence $e^-/H^+/e^-$, the observations forwarded would equally well fit a scheme involving the sequence $e^-/H\cdot$, and at this date we cannot distinguish between those two schemes.
(8) There is a remote possibility that some of the C-4 hydrogen exchange is a consequence of the slow and thermodynamically unfavorable isomerization of the dihydronicotinamide to its 1,6 isomer via the scheme shown.



Experiments are in progress to test this hypothesis.

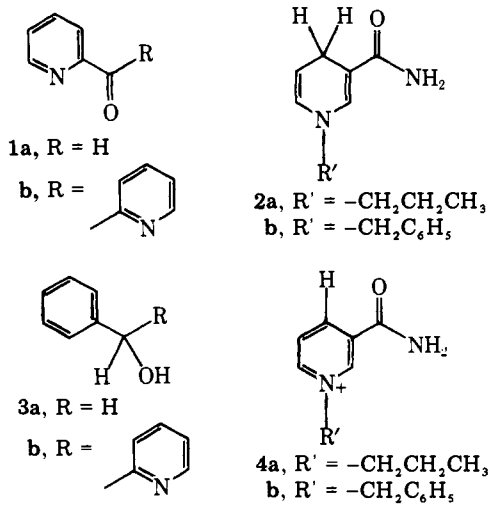
(9) Kosower, E. M.; Tenerstein, A.; Swallow, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 6127.
(10) Kosower, E. M.; Tenerstein, A.; Burrows, H.; Swallow, A. J. *J. Am. Chem. Soc.* **1978**, *100*, 5185.
(11) This, of course, excludes those reactions where the product undergoes facile proton exchange with the solvent.

Paul van Eikeren,* Paul Kenney, Ross Tokmakian
Department of Chemistry, Harvey Mudd College
Claremont, California 91711
Received July 2, 1979

Models for NADH Coenzymes. Reactions of 2-Carboxyl-Substituted Pyridines with *N*-Alkyl-1,4-dihydronicotinamides

Sir:

The reductions of carbonyls by *N*-alkyl-1,4-dihydronicotinamides have been extensively studied[1-3] as model reactions for the enzyme-catalyzed reduction of a carbonyl by NADH. Since we previously observed that the rate of reduction of trifluoroacetophenone by *N*-propyl-1,4-dihydronicotinamide[4] was markedly accelerated by the inclusion of water in the reaction mixture,[5] we decided to study the reactions of 2-carboxyl-substituted pyridines (**1**) with *N*-alkyldihydronicotinamides (**2**) in aqueous solution as model reactions for NADH



dependent redox processes. In this communication, we report that the reaction of 2-carboxyl-substituted pyridines with *N*-alkyldihydronicotinamides leads to the formation of three products: the expected redox products, the primary hydration product of the dihydronicotinamides, and a 1:1 adduct of the carbonyl and dihydronicotinamide to which one molecule of water has been added. The course of the reaction is dependent on the steric hinderance between reactants and the reaction conditions. The implications of the results on the reported disparity between the measured kinetic and partitioning isotope effect in several model systems are discussed.

Pyridine-2-carboxaldehyde (**1a**) reacts rapidly with *N*-propyldihydronicotinamide (**2a**) in aqueous solution. The kinetics of the reaction were studied spectrophotometrically in 0.1 M aqueous (pH 9.9) carbonate buffered solvent or solvent mixtures maintained at constant temperature in a thermostated cell holder. In the presence of an excess of pyridine-2-carboxaldehyde (**1a**), the disappearance of *N*-propyldihydronicotinamide (**2a**) monitored at 360 nm, follows first-order kinetics for over 4 half-lives. The variation in the pseudo-first-order rate constant, k_{obsd}, with increasing concentrations of pyridine-2-carboxaldehyde is linear and provides the self-decomposition rate, k_{dec}, of **2a** and the second-order rate constant, k, for the reaction of **1a** and **2a** through the following equation:

$$k_{obsd} = k_{dec} + k[\mathbf{1a}]$$

Rate constants were evaluated by computer via the method of least squares.

The rate of the reaction of **1a** and **2a** is very sensitive to the nature of the solvent or solvent mixture. The reaction occurs in methanol at 35.0 ± 0.1 °C with a second-order rate constant of (6.0 ± 0.8) × 10^{-3} M^{-1} min^{-1}. Changing the solvent to water increases the rate of the reaction by a factor of over 7000 as shown by a second-order rate constant of 43.4 ± 1.2 M^{-1} min^{-1} at the same temperature. The measured value of k_{dec} in aqueous solution was 0 in accord with the known stability of dihydronicotinamides at high pH.[6] To determine if the C–H bond at the 4 position of the dihydronicotinamide was broken in the rate-determining step, the primary isotope effect for the reaction was measured. A comparison of the second-order rate constants at 30.7 ± 0.1 °C in 0.1 M (pH 9.9) carbonate buffer for the reaction of **1a** with *N*-propyldihydronicotinamide (k_{HH} = 32.5 ± 0.9 M^{-1} min^{-1}) and [4-^{2}H]-*N*-propyldihydronicotinamide (k_{HD} = 31.4 ± 1.2 M^{-1} min^{-1}) indicates no significant primary isotope effect.

The course of the reaction of pyridine-2-carboxaldehyde (**1a**) and *N*-alkyldihydronicotinamides (**2**) in aqueous solution was also monitored by high pressure liquid chromatography (HPLC). In a typical experiment, preequilibrated buffered solutions of pyridine-2-carboxaldehyde (**1a**) and [7-^{14}C]-*N*-benzyldihydronicotinamide (**2b**) are mixed and maintained at 40.0 ± 0.1 °C. Aliquots are removed at various times and the reaction components separated by a high pressure liquid chromatograph coupled to a UV detector. Peaks corresponding to the reactants are quantified by their peak heights and the use of standard curves. All peaks are individually collected and assayed for radioactivity by liquid scintillation counting. Observed counts per minute are corrected for quenching by the use of an external standard.

Time dependent HPLC studies of the above reaction demonstrated the complete consumption of pyridine-2-carboxaldehyde (**1a**, by UV detection) and *N*-alkyldihydronicotinamide (**2**, by UV and radiochemical detection) during the course of the reaction. A typical run is shown in Figure 1. HPLC analysis, however, failed to demonstrate the production of the expected reduction product, the carbinol (**3a**). Instead, HPLC analysis (UV and radiochemical detection) demonstrated the appearance of three other products during the course of the reaction. Radiochemical measurements demonstrated that these three products account for >90% of the *N*-alkyldihydronicotinamide consumed in the course of the reaction. All these products showed a UV maximum at 290 nm. The peaks

0002-7863/79/1501-7406$01.00/0