

Charge Separation on the Micelle Surface as Evidence for a Multi-step Hydrogen Transfer Mechanism in NADH Model Reduction

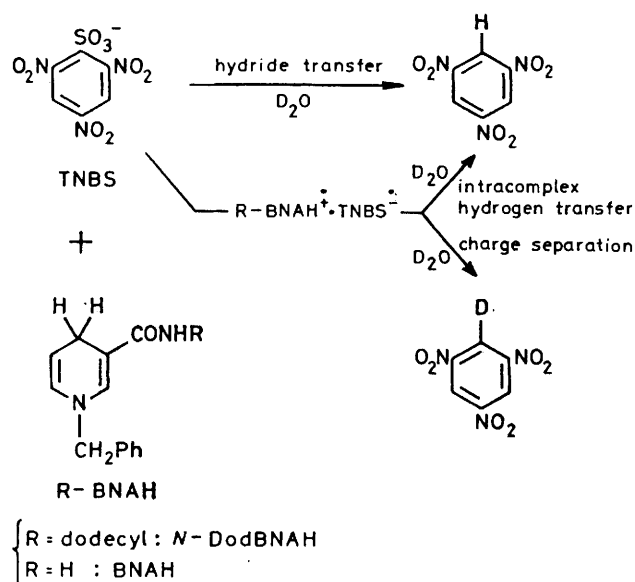
Seiji Shinkai,* Takaharu Tsuno, and Osamu Manabe

Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852, Japan

Reductive desulphonation of 2,4,6-trinitrobenzenesulphonate by *N*-dodecyl-1-benzyl-1,4-dihydronicotinamide bound to the sodium dodecyl sulphate micelle in D₂O solution gave 1,3,5-trinitrobenzene which contained 4.6—5.1% of deuterium; the result indicates that the reaction proceeds through multi-step hydrogen transfer *via* a radical ion-pair intermediate and the micelle surface is capable of dissociating the radical ion pair.

In 1957, Abeles *et al.*¹ proposed in their pioneering study that the hydrogen transfer from NADH model compounds to substrates proceeds through one-step hydride transfer. The hydride transfer mechanism has since been supported by a

number of NADH model studies.^{2,3} However, other reports have proposed multi-step $e + H^\bullet$ (or $e + H^+ + e$) transfer, evidence for the multi-step hydrogen transfer mechanism being the fact that the kinetic deuterium isotope effect (k_H/k_D) is



Scheme 1

significantly smaller than the H/D isotopic ratio in the product ($Y_{\text{H}}/Y_{\text{D}}$).^{4,5} This result has been accommodated by assuming that there is at least one intermediate along the reduction pathway. There are a few examples, however, in which $k_{\text{H}}/k_{\text{D}}$ values are in accord with $Y_{\text{H}}/Y_{\text{D}}$ within experimental error.^{6,7} Furthermore, the first report concerning the discrepancy between $k_{\text{H}}/k_{\text{D}}$ and $Y_{\text{H}}/Y_{\text{D}}$ ⁴ was corrected later by van Eikeren *et al.*^{7,8} Other evidence is associated with the observation of e.s.r. signals and the spin-trapping of radical intermediates in NADH model reduction systems.⁹ However, Hood *et al.*¹⁰ reported that a free-radical intermediate is highly unlikely and radicals detected by e.s.r. spectroscopy may be artefacts. Thus, the hydrogen transfer mechanism for reduction of NADH model compounds is still controversial.

It occurred to us that more direct evidence could be obtained by employing a charge-separation phenomenon on a micelle surface which has been developed in investigations mimicking the initial step in photosynthesis.¹¹ If the reaction proceeds through one-step hydride transfer, a hydrogen atom of the NADH model compound must be transferred directly to a substrate (S) under all reaction conditions. On the other hand, if the reaction proceeds through multi-step hydrogen transfer via a radical ion-pair intermediate ($\text{NADH}^{\bullet+} \cdot \text{S}^{\bullet-}$), $\text{NADH}^{\bullet+}$ would be tightly associated with the anionic micelle while $\text{S}^{\bullet-}$ would be excluded from the micelle surface owing to

electrostatic repulsion between the negative charges. The micelle surface would thus facilitate the dissociation of the ion pair, resulting in the incorporation of a solvent deuterium atom into the radical-anionic substrate isolated in the bulk D_2O phase. We tested the possibility through the reductive desulphonation of 2,4,6-trinitrobenzenesulphonate (TNBS) to 1,3,5-trinitrobenzene (TNB) by *N*-dodecyl-1-benzyl-1,4-dihydronicotinamide (*N*-DodBNAH) in D_2O solution (Scheme 1).

It has been established by Kurz and Frieden³ that the dihydronicotinamide reduction of TNBS proceeds quantitatively. We carried out the reaction at 30 °C and pH 8.2 in anaerobic D_2O (99.9% purity) solution. TNB was isolated by t.l.c. and its deuterium content was estimated by mass spectrometry. The isolated yields of TNB (NADH model base) were 45–60%. We first showed that solvent deuterium is not incorporated into TNB in D_2O or D_2O containing 20 mM sodium dodecyl sulphate (SDS) under the conditions used. The TNB recovered after 2 h from the D_2O solutions gave a ratio of $M^+ + 1$ to M^+ ions in the mass spectrum of $8.1 \pm 0.2\%$, which is in good accord with the natural abundance value (7.91%). Entries 1 and 2 in Table 1 show that no deuterium is incorporated into the product TNB in the reaction of 1-benzyl-1,4-dihydronicotinamide (BNAH) with TNBS; apparently, hydrogen is transferred directly from BNAH to TNBS. On the other hand, when TNBS was reduced by the hydrophobic *N*-DodBNAH in the presence of the SDS micelle, the recovered TNB contained 4.6–5.1% of deuterium. The reactions were repeated three times and the resultant mass spectral data were found to be reproducible within experimental errors (see Table 1). Incorporation of solvent deuterium also occurred in the presence of the nonionic surfactant (Brij-35) or the anionic membranous surfactant ($2\text{C}_{12}\text{SO}_3\text{Na}$),¹² but the deuterium contents were lower than those obtained in the SDS micelle.

The foregoing results suggest that the micelle surface is capable of separating, although partially, micelle-bound *N*-DodBNAH^{•+} from hydrophilic TNBS^{•-} (dianion) and, as a result, solvent deuterium is incorporated into TNBS^{•-} in the bulk D_2O solution. This finding indicates that in the dihydronicotinamide reduction of TNBS, there is a route to products which is not *via* a hydride transfer and is probably the multi-step $e + \text{H} \cdot$ (or $e + \text{H}^+ + e$) mechanism. The low deuterium incorporation could be rationalised in two ways: (i) the hydride transfer mechanism competes successfully with the multi-step mechanism *via* a radical ion pair or (ii) the reaction exclusively proceeds through the multi-step mechanism but the intracomplex (*N*-DodBNAH^{•+} · TNBS^{•-}) hydrogen transfer occurs more efficiently than the charge separation. Further investigation is needed to provide a clear answer to this question.

Table 1. Contents of deuterium in recovered 1,3,5-trinitrobenzene^a

Entry	NADH model	Additive (conc./mM)	$(M^+ + 1)/M^+$ (%) ^d	% Deuteriated 1,3,5- trinitrobenzene
1	BNAH	None	8.3 ± 0.3	0 (<0.4)
2	BNAH	SDS(20)	8.1 ± 0.2	0 (<0.2)
3	<i>N</i> -DodBNAH	SDS(20)	13.0 ± 1.0	5.1
4	<i>N</i> -DodBNAH ^b	SDS(20)	12.5 ± 0.5	4.6
5	<i>N</i> -DodBNAH	Brij-35(20)	10.0 ± 0.5	2.1
6	<i>N</i> -DodBNAH ^b	$2\text{C}_{12}\text{SO}_3\text{Na}(1.0)^c$	11.4 ± 0.4	3.5

^a 30 °C, pH 8.2 with 0.02 M *N*-ethylmorpholine, 2 h in a N_2 stream, 99.9% D_2O , $[\text{NADH model}] = 1.4 \times 10^{-2}$ M, $[\text{TNBS}] = 2.8 \times 10^{-2}$ M.

^b pH 8.2 with 0.02 M phosphate. ^c $\text{Me}[\text{CH}_2]_{11}\text{O}_2\text{CCH}_2\text{CH}(\text{SO}_3^- \text{Na}^+)\text{CO}_2[\text{CH}_2]_{11}\text{Me}$. ^d Ratio of ion intensities in the mass spectrum.

We thank Professor T. Matsuo (Kyushu University) for helpful discussions on the charge separation in the micellar systems.

Received, 15th February 1982; Com. 161

References

- 1 R. H. Abeles, R. F. Hutton, and F. H. Westheimer, *J. Am. Chem. Soc.*, 1957, **79**, 712.
 - 2 D. J. Creighton and D. S. Sigman, *J. Am. Chem. Soc.*, 1971, **93**, 6314; F. M. Martens, J. W. Verhoeven, R. A. Gase, U. K. Pandit, and Th. J. de Boer, *Tetrahedron*, 1978, **34**, 443; J. W. Bunting and S. Sindhuatmadja, *J. Org. Chem.*, 1981, **46**, 4211.
 - 3 L. C. Kurz and C. Frieden, *J. Am. Chem. Soc.*, 1975, **97**, 677; 1980, **102**, 4198; *Biochemistry*, 1977, **16**, 5207.
 - 4 J. J. Steffens and D. M. Chipman, *J. Am. Chem. Soc.*, 1971, **93**, 6694.
 - 5 D. J. Creighton, J. Hajdu, G. Mooser, and D. S. Sigman, *J. Am. Chem. Soc.*, 1973, **95**, 6855; J. Hajdu and D. S. Sigman, *ibid.*, 1976, **98**, 6060; A. Ohno, H. Yamamoto, and S. Oka, *J. Am. Chem. Soc.*, 1981, **103**, 2041; A. Ohno, T. Shio, H. Yamamoto, and S. Oka, *ibid.*, 1981, **103**, 2045; S. Shinkai, T. Ide, H. Hamada, O. Manabe, and T. Kunitake, *J. Chem. Soc., Chem. Commun.*, 1977, 848; S. Shinkai, T. Tsuno, and O. Manabe, *Chem. Lett.*, 1981, 1203; A. Ohno, H. Yamamoto, T. Okamoto, S. Oka, and Y. Ohnishi, *ibid.*, 1978, 65; for comprehensive reviews, see R. J. Kill and D. A. Widdowson, *Bioorg. Chem.*, 1978, **4**, 239; D. S. Sigman, J. Hajdu, and D. J. Creighton, *ibid.*, p. 385.
 - 6 A. K. Colter, G. Saito, F. J. Sharom, and A. P. Hong, *J. Am. Chem. Soc.*, 1976, **98**, 7833; *Can. J. Chem.*, 1977, **55**, 2741.
 - 7 P. van Eikeren, D. L. Grier, and J. Eliason, *J. Am. Chem. Soc.*, 1979, **101**, 7405.
 - 8 D. M. Chipman, R. Yaniv, and P. van Eikeren, *J. Am. Chem. Soc.*, 1980, **102**, 3244.
 - 9 A. Ohno and N. Kito, *Chem. Lett.*, 1972, 369; C. C. Lai and A. K. Colter, *J. Chem. Soc., Chem. Commun.*, 1980, 1115.
 - 10 R. A. Hood, R. H. Prince, and K. A. Rubinson, *J. Chem. Soc., Chem. Commun.*, 1978, 300.
 - 11 K. Kano, K. Takuma, K. Ikeda, D. Nakajima, Y. Tsutsui, and T. Matsuo, *Photochem. Photobiol.*, 1978, **27**, 695; M. S. Tunuli and J. H. Fendler, *J. Am. Chem. Soc.*, 1981, **103**, 2507; P.-A. Brugger, P. P. Infelta, A. M. Braun, and M. Grätzel, *ibid.*, 1981, **103**, 320.
 - 12 T. Kunitake, *J. Macromol. Sci., Chem.*, 1980, **A13**, 587.
-