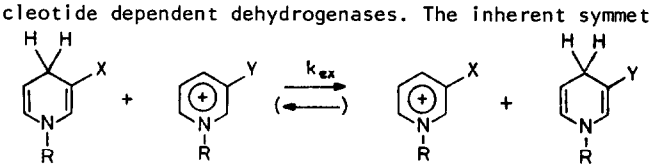
Tetrahedron Letters,Vol.23,No.5,pp 565-568,1982 0040-4039/82/050565-04$03.00/0
Printed in Great Britain ©1982 Pergamon Press Ltd.

INTRAMOLECULAR REDOX SYSTEMS RELATED TO NAD/NADH, PART 1. A KINETIC STUDY OF INTRAMOLECULAR HYDRIDE EXCHANGE BY ^{1}H-SPIN SATURATION TRANSFER

W. van Gerresheim, C. Kruk and J.W. Verhoeven*
Laboratory for Organic Chemistry, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

Abstract: Intramolecular hydride transfer between 1,4-dihydronicotinamide and nicotinamidium moieties connected by a polymethylene chain $-(CH_2)_n-$ is studied by the ^{1}H-spin saturation transfer technique. For n=3 an effective molarity EM=210 is found for the 4,4'-hydride exchange which is not accompanied by the competing 4,6' and solvent exchange processes reported in related intermolecular reactions.

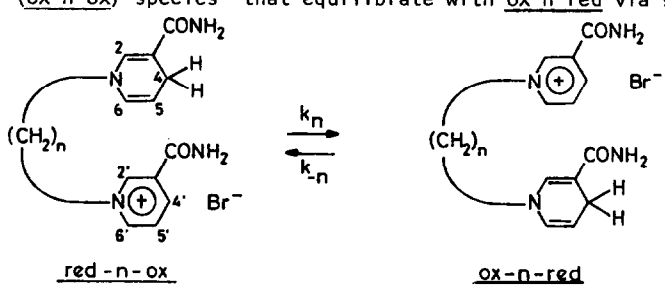
Hydride exchange (cf. Scheme-1) between similar[1-4] or identical[3,5,6] pyridinium ions epitomizes the plethora of reactions studied as models for hydride transfer mediated by pyridine-(di)nucleotide dependent dehydrogenases. The inherent symmetry of degenerate hydride exchange (X=Y) seems to warrant symplifying features[5,6] in a full description of the reaction path, but has made elaborate isotopic labeling and detection techniques imperative in kinetic studies.



Scheme-1.

We now report for the first time that intramolecular hydride exchange (cf. Scheme-2) between nicotinamidium ions connected by a simple polymethylene chain occurs at a rate which enters the time domain amenable to a direct kinetic study by magnetic resonance techniques[7].

Solutions containing the intramolecular redox systems depicted in Scheme-2 were obtained by mixing equivalent amounts of the appropriate doubly reduced (red-n-red) and doubly oxidized (ox-n-ox) species[8] that equilibrate with ox-n-red via slow intermolecular hydride transfer (eqn. 1).



red-n-ox ox-n-red

Scheme-2. Intramolecular hydride exchange processes studied (n=2,3,4).

As evident from the ^{1}H-chemical shift data compiled in Table-1, formation of ox-n-red not only leads to a marked shift of the methylene protons but also to a general upfield shift of the ring protons. The latter phenomenon may be attributed to mutual shielding effects since the pi-systems of ox-n-red are prone[1,9] to enter into weak charge-transfer type interactions.

Although such interactions may also be partly responsible for the significant deviation of K_n from unity, destabilization of ox-n-ox by electrostatic repulsion[8] suffices to explain the rapid increase of K_n for smaller values of n.

(1) ox-n-ox + red-n-red $\overset{K_n}{\rightleftharpoons}$ 2 ox-n-red $\quad K_2 > 25,\ K_3 = 3.1,\ K_4 = 1.4$

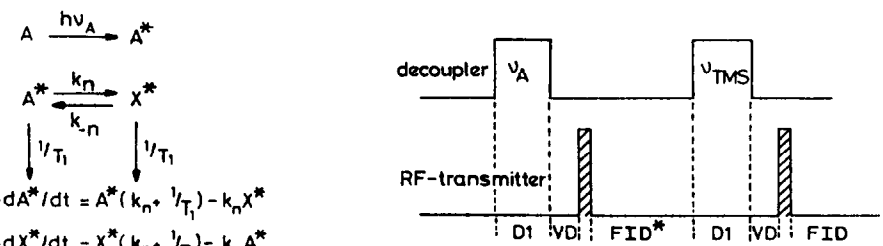
The discrete and sharp ^{1}H-NMR signals for the three species in the equilibrium mixtures (cf. Table-1) prove that neither inter- nor intramolecular hydride exchange occurs at a rate comparable to the NMR frequency difference between chemically related sites.

Table-1: Chemical shift data, ppm rel. to TMS (DMSO-$^2H_6$, 25°C, 250 MHz).

	H-2	H-4	H-5	H-6	H-2'	H-4'	H-5'	H-6'	red-CH_2	ox-CH_2	red-C-CH_2	ox-C-CH_2
ox-2-ox					9.67	9.06	8.33	9.22		5.35		
ox-3-ox					9.65	9.03	8.33	9.36		4.89		2.81
ox-4-ox					9.59	8.98	8.33	9.27		4.77		2.05
red-2-red	6.90	2.86	4.61	5.91					3.20			
red-3-red	6.86	2.97	4.62	5.87					3.09		1.69	
red-4-red	6.89	2.98	4.60	5.89					3.13		1.45	
ox-2-red	6.78	2.85	4.55	5.80	9.52	8.95	8.28	9.12	3.70	4.78		
ox-3-red	6.87	2.89	4.58	5.87	9.53	8.95	8.17	9.16	3.26	4.72	(—— 2.27 ——)	
ox-4-red	6.89	2.98	4.60	5.89	9.55	8.96	8.27	9.24	3.16	4.72	1.50	1.96

Irradiation however of the various proton signals of ox-n-red (n=3,4) selectively decreases the intensity of signals chemically related to those irradiated via intramolecular 4,4'-hydride transfer.

The theory and methodology of the spin saturation transfer underlying this effect have been documented extensively[10,11]. Scheme-3 summarizes the kinetic equations for chemical transfer ($k_n = k_{-n}$) of spin saturation (indicated by an asterisk) between non-coupled sites A and X with equal spin lattice relaxation times T_1. Furthermore Scheme-3 shows the pulse sequence employed to obtain by difference FT-NMR a time resolved picture of the development of spin saturation at sites A and X after saturation of A for a period D1. Because of their narrow signals and relatively long and equal T_1 values H-2' and H-2 were chosen as A and X respectively. This sequence avoids effects of spectral overlap between the H-2 signals of ox-n-red and red-n-red (cf. Table-1) since the H-2 signal of the latter is nullified in a difference FT-NMR spectrum.



Scheme-3

Two methods were employed[10,11] to extract the value of k_n from the time resolved spin saturation data.

In method-1 the time evolution of the sum and difference of spin saturation at sites A and X is

followed (VD=0.001-10 s). These are given[11] by (2) and (3):

$$(2) \quad \ln(A^* + X^*) = -t/T_1 + \ln(A_0^* + X_0^*)$$

$$(3) \quad \ln(A^* - X^*) = -(1/T_1 + 2k_n)t + \ln(A_0^* - X_0^*)$$

This determines both T_1 and k_n from a single series of measurements. In method-2 the value of D1 is chosen long enough (D1=10 s) to reach steady-state conditions. The ratio of spin saturations at A and X measured immediately after D1 is then given by (4):

$$(4) \quad A_0^*/X_0^* = (1/T_1 + k_n)/k_n$$

This allows determination of k_n from a single measurement (i.e. if T_1 is known from a separate measurement[12] or from method-1), which turned out to be an essential requirement for measuring k_4 in the high temperature region (T > 340 K) where slow decomposition prevents application of method-1.

Kinetic data thus determined at a number of temperatures are summarized in Table-2.

Table-2: Kinetic data and activation parameters for intramolecular 4,4'-hydride exchange in ox-n-red (solvent DMSO-$^2H_6$).

n	T (K)	$1/T_1$ (s^{-1})	k_n (s^{-1})	$\Delta H^{\ddagger}_{298}$ (kJ.mol^{-1})	$-\Delta S^{\ddagger}_{298}$ (J.mol^{-1}.K^{-1})
2	363	(1.1)[a]	< 0.04	-	-
3	298	1.40	0.13[b]		
	313	1.15	0.43		
	323	0.90	1.00	56.4 ± 1.5	72.3 ± 1.2
	333	0.88	1.32		
	343	0.93	3.20		
4	348	(1.1)[a]	0.22[c]		
	353	(1.1)	0.29		
	358	(1.1)	0.42	79.2 ± 4.3	31.7 ± 3.5
	363	(1.1)	0.71		

a) Extrapolated from values determined at T < 340 K; b) Method-1 (cf. text); c) Method-2 (cf. text).

For n=2 no spin saturation transfer could be detected even at the highest temperature (363 K) investigated. For n=3 and 4 the kinetic data fit good linear Arrhenius plots represented by (5) and (6) respectively:

$$(5) \quad \ln(k_3/s^{-1}) = (21.8\pm0.6) - (58.8\pm1.5 \text{ kJ.mol}^{-1})/RT \quad \text{(corr. coeff. r = .992)}$$

$$(6) \quad \ln(k_4/s^{-1}) = (26.6\pm1.7) - (81.7\pm4.3 \text{ kJ.mol}^{-1})/RT \quad \text{(corr. coeff. r = .990)}$$

This leads to the Eyring activation parameters indicated in Table-2.

An illuminating impression of the efficiency of the intramolecular exchange process in ox-3-red is achieved upon comparison with the kinetics of a closely related intermolecular exchange (cf. Scheme-1). Thus $k_{ex} = 2\times10^{-3}$ $M^{-1}.s^{-1}$ at 313 K for R=benzyl and X=Y=$CONH_2$ in an acetonitrile-0.1 M aqueous phosphate buffer (pH 8.6) solvent mixture (1:3 v/v) was reported[5]. This implies an effective molarity[13] EM=210 for n=3 (for n=4 EM=4 by extrapolation of (6)), which unequivocally identifies the symmetric and parallel face to face orientation of the pyridine rings enforced by a trimethylene chain[14] as a favoured geometry for hydride exchange.

As an apparent consequence of these conformational constraints the intramolecular hydride ex-

change displays extreme regiospecificity. While intermolecular exchange (R=Me, X=Y=$CONH_2$ cf. Scheme-1) was reported[15] to lead to an eventual equilibrium mixture of the 1,4- and 1,6-dihydro isomers (9:2) due to competitive 4,6'-hydride transfer, no trace of such isomerization could be detected for ox-n-red (n=3,4) even after periods of reaction which allow for a number of intramolecular exchange cycles that exceeds the number of exchange cycles used in the intermolecular process[5,6,15] by several orders of magnitude

Furthermore a loss of ^{3}H-4 label to the solvent was found[6] to occur with a rate ~50 times slower than the 4,4'-exchange during the intermolecular process (R=benzyl, X=Y=$CONH_2$). It could not be excluded, however, that this loss of label occurs via a route involving transfer of the label to C-6' followed by the well known solvent exchange of H-6'.

Reaction of ox-3-red for 24 hrs at 313 K in CH_3OH-$^2H_4$ leads to no detectable (< 10%) incorporation of ^{2}H at C-4. This implies that the chance for a hydrogen nucleus to be exchanged with solvent protons "on flight" between C-4 and C-4' is less than 1:50,000!

In conclusion the present data not only yield direct information regarding the geometry of the transition-state for 4,4'-hydride exchange but also strongly militate against the formation of any intermediate species prone to undergo proton exchange with the solvent. On the other hand the conformational and thermodynamic data obtained, seem compatible with formation of a -short lived- symmetrical μ-hydrido bridged intermediate, analogous to those recently observed[16] in medium-sized cyclic carbocations. This possibility and the applicability of magnetic resonance techniques as a tool for direct determination of the kinetics of hydride transfer in other systems related to the NAD/NADH couple are presently under investigation.

REFERENCES

1. J. Ludowieg and A. Levy, Biochemistry 3, 373 (1964).
2. J.B. Jones and K.E. Taylor, Can. J. Chem. 54, 2974 (1976).
3. T.J. van Bergen, T. Mulder, R.A. van der Veen and R.M. Kellogg, Tetrahedron 34, 2377 (1978).
4. A. Ohno, H. Yamamoto and S. Oka, J. Am. Chem. Soc. 103, 2041 (1981).
5. P. van Eikeren and D.L. Grier, J. Am. Chem. Soc. 99, 8057 (1977).
6. P. van Eikeren, P. Kenney and R. Tokmakian, ibid. 101, 7402 (1979).
7. Intramolecular hydride exchange between other functional groups has also been studied by dynamic NMR techniques: a) M. Saunders and J.J. Stofko, J. Am. Chem. Soc. 95, 252 (1973). b) G.A. Craze and I. Watt, J. Chem. Soc., Perkin Trans. II 175 (1981).
8. J.H. Craig, P.C. Huang, T.G. Scott and N.J. Leonard, J. Am. Chem. Soc. 94, 5872 (1972).
9. G. Cilento and P. Tedeschi, J. Biol. Chem. 236, 907 (1961).
10. F.A.L. Anet and A.J.R. Bourn, J. Am. Chem. Soc. 89, 760 (1967).
11. F.W. Dahlquist, K.J. Longmuir and R.B. du Vernet, J. Magn. Res. 17, 406 (1975) and references cited therein.
12. Measurement of spin relaxation times and verification of their equality for H-2 and H-2' was accomplished by standard techniques.
13. A.J. Kirby, "Advances in Physical Organic Chemistry", 17, 183 (1980).
14. N.J. Leonard, Acc. Chem. Res. 12, 423 (1979).
15. H. Minato, T. Ito and M. Kobayashi, Chem. Lett. 13 (1977).
16. R.P. Kirchen, K. Ranganayakulu, B.P. Singh and T.S. Sorensen, Can. J. Chem. 59, 2173 (1981).

(Received in UK 1 December 1981)