Asymmetric Intercoenzyme Hydrogen Transfer between NADH Model Compounds and Flavins

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A chiral flavin [(*S*)-3-methyl-10-(1′-hydroxy-3′-methylbutan-2′-yl)isoalloxazine, (**1b**)] has been synthesised: hydrogen transfer to (**1b**) and tetra(*O*-acetyl)riboflavin from chiral NADH model compounds occurred in an asymmetric manner only in the presence of high concentrations of $Mg(ClO_4)_2$.

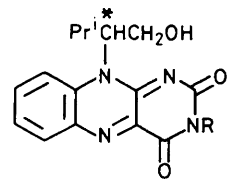
In the past decade, asymmetric reduction of substrates with carbonyl groups by optically active NADH model compounds has been widely investigated.[1] To the best of our knowledge, however, no precedent exists for asymmetric hydrogen transfer in a model system from NADH to flavin, one of the most important intercoenzyme reactions. Moreover, a flavin bearing a chiral substituent has never been synthesised. The reaction in the nonenzymic system usually appears to be first-order in both the NADH model compound and the flavin but follows Michaelis–Menten type saturation kinetics at high reactant concentrations because of the necessary face-to-face orientated stacking in the reaction.[2,3] The existence of the Michaelis-complex-like intermediates similar to those in the enzymic system suggests that the model reaction may be used to design an asymmetric hydrogen transfer system.

We have synthesised an optically active flavin (**1b**)† and examined the ability of it and that of tetra(*O*-acetyl)riboflavin (**2**), a derivative of natural, optically active flavin, in the asymmetric oxidation. As NADH model compounds we used (*R*)- and (*S*)-*N*-α-methylbenzyl-1-propyl-1,4-dihydronicotinamide (**3**) and (*R*)- and (*S*)-1-α-methylbenzyl-1,4-dihydronicotinamide (**4**).

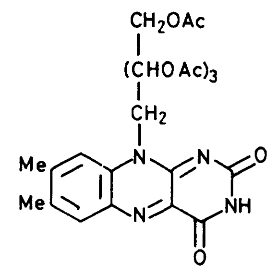
Compound (**1b**) has several novel properties that are quite different from those of conventional flavins. In contrast to the poor solubility of conventional flavins, (**1b**) and (*S*)-10-(1′-hydroxy-3′-methylbutan-2′-yl)isoalloxazine (**1a**) are very soluble in acetic acid, methanol, ethanol, or acetonitrile, so that these solvents could not be used for recrystallisation. The 10-substituent was easily eliminated either by acid catalysis or by visible light irradiation to precipitate 3-methylalloxazine and alloxazine, respectively. Examination of the Corey–Pauling–Koltun models suggested that (**1b**) and (**1a**) with a secondary carbon atom attached to N(10), have significant steric crowding around N(10) and that the substituents on the asymmetric carbon atom protrude above and below the isoalloxazine plane. Therefore, the efficient face-to-face stacking association seems to be energetically disfavoured. This observation may be an important clue in explaining why natural flavin coenzymes have the ribityl group with a CH_2 group attached to N(10).

Anomalies were also observed in reactivity (Table 1). When compared with the reactivities of 10-ethylisoalloxazine (**5**) with no steric hindrance at N(10), (**1a**) gave smaller rate constants for oxidations of 1-benzyl-1,4-dihydronicotinamide (BNAH) and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (BCQH) which proceed *via* transition states with face-to-face orientations.[2,3] In contrast, (**1a**) is more reactive for oxidations of thiols which proceed *via* 4a-adducts. One can conclude, therefore, that the reactions involving the stacking association as an obligatory path are selectively suppressed in (**1a**).

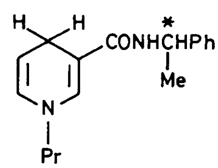
† (**1**) was synthesised from *o*-fluoronitrobenzene and L-valinol as in Scheme 1 {m.p. 235.5—237.0 °C, $[\alpha]_D^{25}$ −35.0° (methanol)}.



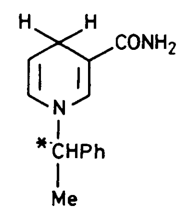
(**1**)
a; R = H
b; R = Me



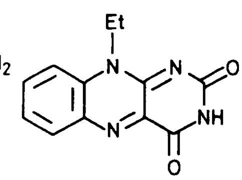
(**2**)



(**3**)



(**4**)



(**5**)

Table 1. Pseudo first order rate constants ($10^4.k_1'$, s^{-1}) for oxidations by (**1a**) and (**5**).[a]

Reactant (mM)	$10^4.k_1'$ (**1a**)	$10^4.k_1'$ (**5**)	Rate ratio of (**1a**) *vs.* (**5**)
BNAH (0.0956)	1.35	9.40	0.114
BCQH (0.0995)	0.093	0.442	0.210
$HS[CH_2]_4SH$ (1.07)	4.38	1.32	3.32
$HO[CH_2]_2SH$ (10.0)	0.0391	0.0172	2.27

[a] 30 °C, pH 8.66 with 0.075 M borate buffer, [flavin] = (4.8—5.1) × 10^{-5} M.

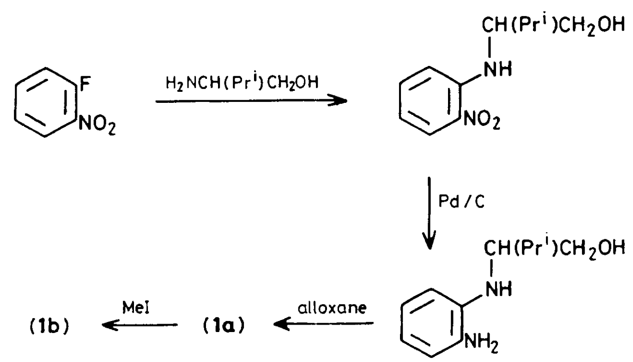
We first attempted the asymmetric hydrogen transfer in an aqueous system but this failed. We then studied it in acetonitrile {$[H_2O]$ = (1—2) × 10^{-2} M} in the presence of $Mg(ClO_4)_2$, $KClO_4$, or Bu_4NBr (Table 2).‡ In the presence of Bu_4NBr no asymmetric discrimination was observed. Similarly, the addition of $KClO_4$ (2 × 10^{-3} M) resulted in no asymmetric effect. In the presence of a low concentration of $Mg(ClO_4)_2$, a small difference in the rate constants appeared between (*R*)- and (*S*)-dihydronicotinamides. The difference apparently increased at higher $Mg(ClO_4)_2$ concentrations and

‡ In the reaction with (**3**), k_2 increases with increasing $Mg(ClO_4)_2$ concentration, whereas in the reaction with (**4**) it decreases with increasing $Mg(ClO_4)_2$ concentration. The contrast may be due to different binding positions of the Mg^{2+} ion.

Table 2. Second order rate constants (k_2, $mol^{-1}\ dm^3\ s^{-1}$) for the reactions of (**1b**) or (**2**) with NADH model compounds.[a]

	k_2 for (**3**)			k_2 for (**4**)		
Flavin–additive (mM)	*R*	*S*	$k_{2,R}/k_{2,S}$	*R*	*S*	$k_{2,R}/k_{2,S}$
(**1b**)–$Mg(ClO_4)_2$ (0.2)	0.33	0.32	1.03	0.22	0.24	0.92
(**1b**)–$Mg(ClO_4)_2$ (100)	1.14	1.05	1.09	0.077	0.098	0.79
(**2**)–$Mg(ClO_4)_2$ (0.2)	0.89	0.86	1.03	0.42	0.43	0.98
(**2**)–$Mg(ClO_4)_2$ (100)	3.80	1.99	1.91	0.24	0.18	1.33
(**2**)–Bu_4NBr (100)	0.23	0.24	0.96			

[a] 30 °C, acetonitrile, [flavin] = 5.00×10^{-5}, [NADH model compound] = 1.00×10^{-4} M.



Scheme 1

the reaction between (**2**) and (**3**) gave the greatest ratio ($k_{2,R}/k_{2,S}$ = 1.91).

It is known that dihydronicotinamides form complexes with $Mg(ClO_4)_2$ in acetonitrile (K_c = *ca.* $10^3\ M^{-1}$).[4] We also found flavins formed complexes with $Mg(ClO_4)_2$ in acetonitrile as shown by absorption spectral changes. The absorption maxima of (**1b**) (436), (**2**) (442), and (**5**) (434 nm) in acetonitrile shifted to 423, 433, and 422 nm, respectively, in the presence of 0.1 M $Mg(ClO_4)_2$. No spectral changes were observed in the presence of $KClO_4$ or Bu_4NBr. From the concentration dependence in acetonitrile {$[H_2O] = (1—3) \times 10^{-2}$ M} we estimated the K_c to be 60.9, 124, and 40.8 $mol^{-1}\ dm^3$, respectively. Since the K_c values for dihydronicotinamides are much greater than those for flavins, one can envisage that $Mg(ClO_4)_2$ first binds to the dihydronicotinamides and that flavin–$Mg(ClO_4)_2$ complexes are produced only at higher $Mg(ClO_4)_2$ concentrations. Anyhow, the absence of the asymmetric recognition in aqueous solution and in acetonitrile in the presence of Bu_4NBr and $KClO_4$ shows the importance of Mg^{2+} ion as a 'bridge' in the transition state where hydrogen is transferred asymmetrically from NADH model compounds to flavins.

In conclusion, the present study has established for the first time that asymmetric hydrogen transfer occurs from NADH model compounds to flavins with the aid of Mg^{2+} ion that interacts with both reactants. The lack of chiral recognition in aqueous systems is therefore ascribed to poorer Mg^{2+} complex formation, which is shown by the lack of absorption spectral changes on addition of Mg^{2+}.

Added in proof: Fukuzumi *et al.* (*Chem. Lett.*, 1984, 417) have also reported the flavin–Mg^{2+} interaction in MeCN.

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