Flavin plus 5-Deazaflavin as a Turnover Oxidation System: Model for the Electron Bridge from NAD^+ to Flavin

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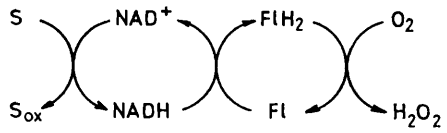
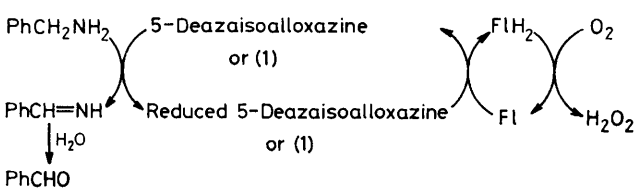
Summary An efficient model system for the electron bridge from NAD^+ to flavin was constructed for the first time by using 5-deazaisoalloxazine and flavin mononucleotide, the yield in the aerobic oxidation of benzylamine to benzaldehyde being 3500%.

NAD^+ and flavin (Fl) are representative redox coenzymes and present in many biological systems. Their roles as oxidation catalysts are somewhat different,[1-3] so that they frequently work co-operatively (Scheme 1). NAD^+ apparently acts as a 'two-electron-carrying shuttle' and has strong oxidising power but is not reoxidised by molecular oxygen, whereas Fl can be classified as a 'one-electron-carrying shuttle' and is rapidly reoxidised by molecular oxygen;[1-3] overall, the substrate (S) is oxidised by molecular oxygen *via* the mediation of the electron bridge from NAD^+ to Fl. Recently, Yoneda *et al.*[4,5] showed that 5-deazaflavin (or 5-deazaisoalloxazine; 'NAD^+ in flavin clothing'[2]) is able to oxidise alcohols and amines in good yields. Shinkai *et al.*[6] reported that 3-hydroxy-*N*-methylacridinium ion (**1**) which is regarded as a more precise NAD^+ model is also able to oxidise alcohols and amines almost quantitatively. However, the reoxidation of reduced 5-deazaflavin or (**1**) by molecular oxygen is so slow[2,6] that these NAD^+ analogues cannot serve as an efficient turnover oxidation catalyst. A high recycling number for a single-compound model system is attained only with 4-deazatoxoflavins.[7] It occurred to us that Fl may mediate in the reoxidation step, and we now report that the combination of 5-deazaisoalloxazine or (**1**) with flavin mononucleotide (FMN) facilitates the



SCHEME 1



SCHEME 2

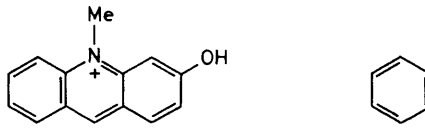
TABLE. Aerobic turnover oxidation of benzylamine to benzaldehyde mediated by NAD^+ analogues plus flavin.[a]

Run	NAD^+ analogue	Flavin (mmol)	ml of $CHCl_3$	mmol of PhCHO	% Yield of PhCHO based on NAD^+ analogue
1	(2)	None	0	0·142	171
2	(2)	(3) (0·209)	0	0·344	414
3	(2)	None	3	0·311	379
4	(2)	FMN (0·209)	3	0·643	775
5	(2)	FMN (0·209)	3[b]	0·374	451
6	(2)	FMN (0·209)	0	2·91	3500
7	(1)	None	0	2·34	2820
8	(1)	FMN (0·209)	0	2·88	3470

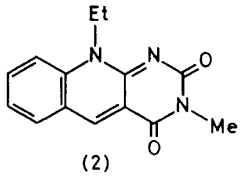
[a] Typical conditions: a mixture of O_2-saturated water (10 ml) and benzylamine (3 ml) containing the NAD^+ analogue (0·083 mmol) was heated at 65—70 °C for 1 day in the dark. Molecular oxygen was slowly bubbled into the solution at a constant rate. [b] Tri-n-octylmethylammonium chloride (0·50 g) was added.

turnover oxidation of benzylamine to benzaldehyde (Scheme 2). This is the first example of the construction of a model of the efficient electron bridge from NAD^+ to Fl.

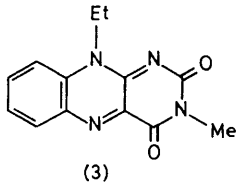
We have used (**1**) or 3-methyl-10-ethyl-5-deazaisoalloxazine (**2**) as an NAD^+ analogue and 3-methyl-10-ethylisoalloxazine (**3**) or FMN as a flavin analogue. Typical conditions are in the Table. The product mixture was diluted (× 10) with water and methanol, heated for 10 min, and analysed by g.l.c. with anisole as internal standard. It was corroborated that the yield determined by the g.l.c. method is equal to that determined by the 2,4-dinitrophenylhydrazine method[5] within the experimental error.



(1)



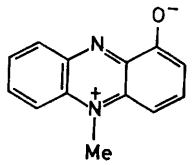
(2)



(3)

The catalytic system containing (**2**) and (**3**) (run 2) gave a yield of benzaldehyde somewhat greater (2·4 fold) than that mediated by (**2**) (run 1). The unexpected inefficiency of this system was attributed to some interaction between (**2**) and reduced (**3**), similar to the interaction between oxidised and reduced flavins in their comproportionation step ($FlH^- + Fl \rightleftharpoons 2\ Fl^{\bullet}$). We thus tried to separate (**2**) from Fl by using a biphasic system (runs 3—5). In the water–chloroform biphasic system, benzylamine and (**2**) mostly reside in the chloroform layer and the water-soluble FMN in the aqueous layer. However, the yield in the (**2**) + FMN system (run 4) was only twice as great as that of (**2**) in the biphasic system (run 3), and the yield was not improved greatly by the addition of a phase-transfer catalyst (run 5).

Surprisingly, we found that the combination of (**2**) with FMN provides an excellent turnover system even in the homogeneous aqueous solution (run 6). The recycle number of 35 is greater by a factor of about 20 than that for (**2**) alone. This clearly indicates that the oxidation of benzylamine by (**2**) + FMN occurs *via* an electron bridge from (**2**) to FMN as shown in Scheme 2. It is not yet clear, however, why the (**2**) + FMN system behaves as a turnover oxidation catalyst, while the (**2**) + (**3**) system does not.



Pyocyanine

Compound (**1**) is isoelectronic with 5-deazaflavin in the same way that pyocyanine is isoelectronic with Fl[8] and is expected to serve as a turnover oxidation catalyst. As shown in the Table, (**1**) led to a recycle number of 28·2 even in the absence of Fl (run 7), indicating that (**1**) is better than (**2**) as a turnover catalyst. The high efficiency of (**1**) is attributed either to its high oxidizibility or to its high sensitivity to molecular oxygen. The recycle number was only slightly improved by the addition of FMN (run 8). The second-order rate constants (k_2) determined spectrophotometrically at 50 °C under anaerobic conditions were: $1{\cdot}1 \times 10^{-5}$ l mol^{-1} s^{-1} for (**1**) and $8{\cdot}6 \times 10^{-5}$ l mol^{-1} s^{-1} for (**2**), the rate constant for (**2**) is thus 8 times greater than that for (**1**). This result, together with the influence of added FMN on the recycle number for (**1**) and (**2**), suggests that the high recycle number for (**1**) is due to its sensitivity to molecular oxygen and that the rate-limiting step for the oxidation by (**2**) is the reoxidation of reduced (**2**) by molecular oxygen.

The present study has thus demonstrated the existence of the model electron bridge from NAD^+ to Fl for the first time, and we believe that higher recycle numbers could be achieved by modifying the structure of (**1**) or (**2**).

(*Received*, 19*th January* 1981; *Com.* 060.)

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