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Coenzyme Models 17. Is 5-Deazaflavin a Model for NAD^+? Comment on the Deviation from Linear Free-energy Relationships

by

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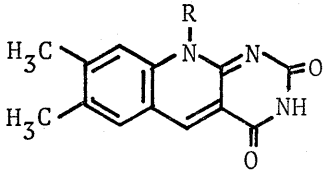
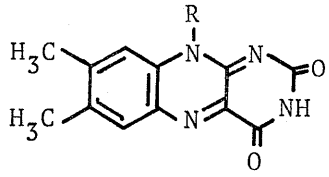
The linear free-energy behavior of 5-deazaisoalloxazine in the reversible adduct formations was evaluated in comparison with that of NAD^+ analogues. It was found that when compared with linear log K(association constant)–log k_f(forward reaction rate constant) relationships for NAD^+ analogues the plots for 5-deazaisoalloxazines deviate to the upper area with no apparent exceptions. The distinct deviation was attributed to the smaller K value relative to the k_f value, which is caused by the electron-donating effect of 1-N^-. Thus, 5-deazaflavin may not be a satisfactory model for NAD^+ from the viewpoint of the linear free-energy relationships.

5-Deazaflavin has been designated as a model of flavin as well as that of NAD^+. It was initially synthesized as a flavin analogue[1,2] and has since been employed as a mechanistic "dummy" in flavin-dependent enzymes and nonenzymatic flavin oxidation reactions.[3–6] In fact, the absorption spectrum is quite similar to that of flavin itself.[2]

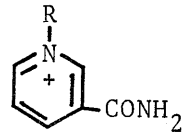


5-Deazaflavin

(5-Deazaisoalloxazine: no methyl group at 7- and 8-positions)



Flavin



NAD^+

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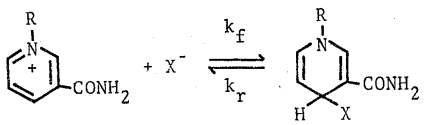
It was found later, however, that 5-deazaflavin has a characteristic essentially different from that of flavin : insensitivity of 1, 5-dihydro-5-deazaflavin to oxygen.[7,8] It was thus proposed that 5-deazaflavin is a model of NAD^+, rather than that of flavin.[9]

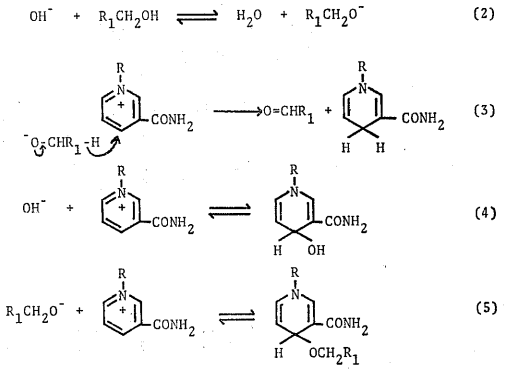
Recently, Yoneda and coworkers[10] demonstrated the facile oxidation of alcohols by 5-deazaisoalloxazines in the presence of hydroxide ion. On the other hand, no precedent exists for the nonenzymatic oxidation of alcohols by NAD^+ analogues.* Although Shirra and Suckling[11] reported the oxidation of benzyl alcohols by *N*-substituted nicotinamide derivatives at 20°C in tetrahydrofuran, they detected only benzaldehydes and production of the corresponding dihydronicotinamides was not established with certainty. It thus seems to us that their claim has still remain-with ambiguity.[12] It would be interesting to specify why 5-deazaisoalloxazine is able to oxidize alcohols and NAD^+ analogue is not, in spite of the proposition that 5-deazaflavin is a model of NAD^+.[9]

It has been established that *N*-substituted nicotinamides and analogues form adducts with nucleophiles such as CN^-, SO_3^{2-}, RS^-, and OH^- under reversible equilibria (Eq. 1).[13]



(1)

Supposing that NAD^+ oxidation of alcohols takes place in the presence of hydroxide ion, it is conceivable that two different types of reactions proceed competitively, hydride transfer[14] from R_1-CH_2O^-(Eq.3) and reversible adduct formation with OH^- or $R_1-CH_2O^-$ (Eqs. 4 and 5).



Since Eqs. 4 and 5 are much faster than Eq. 3, it is readily presumed that NAD^+ analogues have been converted to the corresponding adducts before they accept a hydride from alcohols. Then, why has 5-deazaisoalloxazine a chance to accept a hydride ion[10] in spite of the occurrence of similar adduct formation?[15]

Recently, Shinkai *et al.*[16~18] and Johnson and Smith[19] found that the logarithms of rate and association constants (k_f and $K(=k_f/k_r)$, respectively) for the addition to NAD^+ analogues show good linear relationships. For example, the equation for the addition of cyanide ion to NAD^+ analogues ($X^-=CN^-$ in Eq. 1) is expressed by Eq. 6(at 25°C)[20] or by Eq. 7(at 30°C).[16]

$$\log k_f = 0.55 \log K - 1.45 \quad (6)$$

$$\log k_f = 0.53 \log K - 1.19 \quad (7)$$

On the other hand, a plot for 3, 10-dimethyl-5-deazaisoalloxazine determined by Chan and Bruice[15] (filled circle in Fig. 1) greatly deviated to the upper area from the linear correlation for NAD^+ analogues. The similar upper deviation of 5-deazaisoalloxazine is

*Very recently, Ohnishi and Kitami have reported the oxidation of lithium ethoxide by *N*-substituted nicotinamide derivatives (*Tetrahedron Lett.*, 1978, 4035).

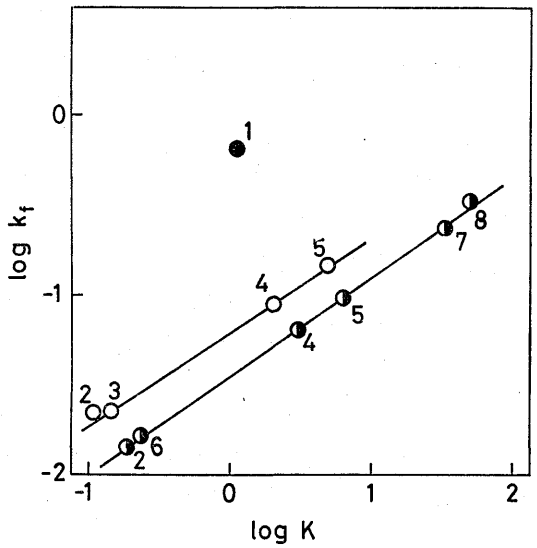


Fig. 1. Log K-log k_f relationship for the addition of cyanide ion to N-substituted nicotinamides. (○) cited from Ref. 16 (at 30°C); (◑) cited from Ref. 20 (at 25°C) ; (●) plot 1 for 3, 10-dimethyl-5-deazaisoalloxazine cited from Ref. 15 (at 30°C). R group of nicotinamide is : 2, propyl ; 3, hexyl ; 4, benzyl ; 5, 2,6-dichlorobenzyl ; 6, methyl ; 7, 4-nitrobenzyl ; 8, 2-chloro -4-nitrobenzyl.

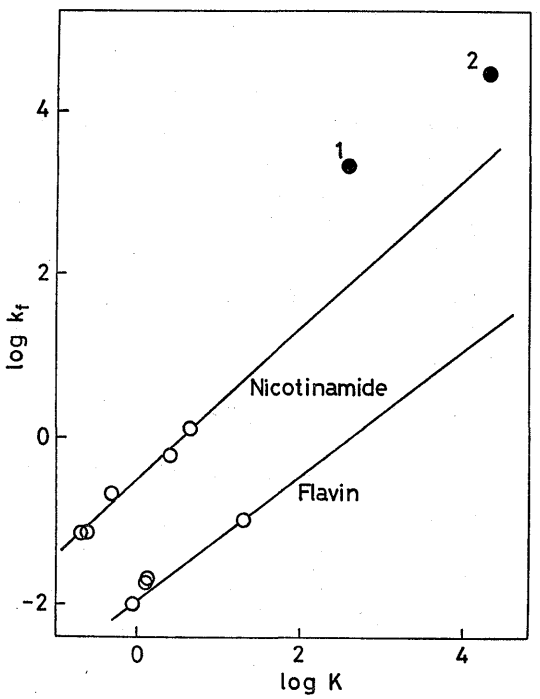


Fig. 2. Log K-log k_f relationship for the addition of sulfite ion to N-substituted nicotinamides[17] and flavins (cited from S. Shinkai, *Makromol. Chem.*, 179, 2637 (1978)). 1, 3,10-dimethyl-5-deazaisoalloxazine ; 2, 3, 10-dimethyl-8-cyano-5-deazaisoalloxazine (both plots are cited from Ref. 15).

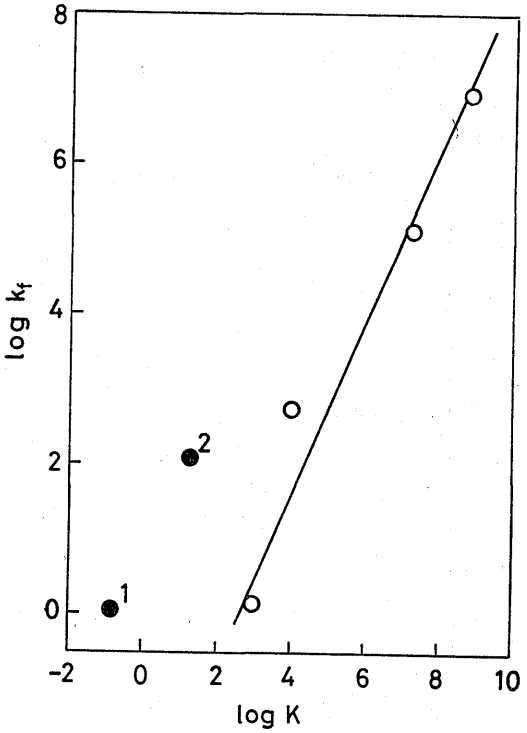
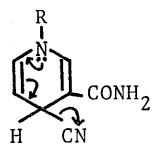


Fig. 3. Log K-log k_f relationship for the addition of hydroxide ion to N-substituted quinolines and acridines (cited from J. W. Bunting and W. G. Meathrel. *Can. J. Chem.*, 51, 1965 (1973) ; *ibid.*, 52, 303 (1974). 1, 3,10-dimethyl-5-deazaisoalloxazine ; 2, 3.10-dimethyl-8-cyano-5-deaza-isoalloxazine (both plots are cited from Ref. 15).

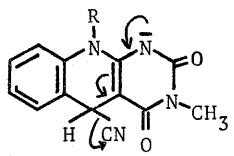
seen in the reaction with sulfite ion (Fig. 2) and hydroxide ion (Fig. 3). Fig. 2 indicates that both rate and association constant for the reaction of NAD^+ analogues are greater than those for flavins[17),18)] and that the affinity of sulfite ion toward 5-deaza-isoalloxazines is further enhanced.

Here, two possibilities arise to account

for the deviation : (i) the forward reaction rate constans (k_f) is greater than unity and (ii) the adduct of 5−deazaisoalloxazine is destabilized relative to that of NAD^+ analogues, resulting in the smaller K value. The case (i) is observed in the reaction between cyanide ion and polymeric N−substituted nicotinamide.[18] The present deviation of 5-deazaisoalloxazines should be rationalized, we believe, in terms of the case (ii). As illustrated below, the driving force for the reverse reaction of NAD-X adduct (8a) is related to lone-pair electrons on 1-N. On the other hand, anionic 1-N^- of 1, 5-dihydro-5-deazaisoalloxazine would participate in the dissociation of 5-deazaisoalloxazine−X adduct(8b) more efficiently, since the basicity is expected to be much stronger than that of 1-N in NAD-X adduct. It may be said that the relatively high electron-donating ability of 1-N^- destabilizes the 5-deazaisoalloxazine-X adduct.

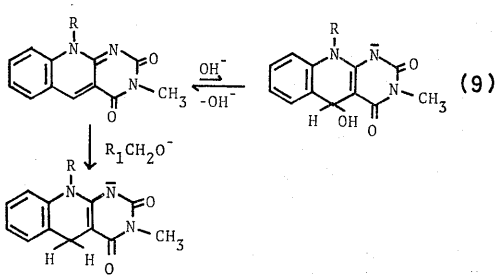


(8a)



(8b)

The foregoing consideration would afford a following reaction scheme which accounts for facile oxidation of alcohols by 5-deazaisoalloxazine.



(9)

Owing to destabilization of the 5-deazaisoalloxazine-OH adduct the concentration of 5-deazaisoalloxazine must be enhanced to a significant extent even in the alkaline pH region and the 5−deazaisoalloxazine would accept a hydride ion from alcohols through a slow, irreversible step.

It is suggested based on the above summary that the NAD^+ analogue which is able to oxidize alcohols must be insensitive kinetically to nucleophiles. Although the proposal is not the case when the hydride transfer is assisted by generale-base catalysis($base^{\delta+}\cdots H\cdots OCHR_1\cdots H^{\delta-}$)[21], there are few precedents for this type of the reaction in which weak general base produces strongly basic hydride ion. It seems, therefore, that the synthetic design of nucleophile-insensitive NAD^+ analogues would lead to the most expeditious method to achieve the NAD^+ model oxidation of alcohols. The synthetic effort is now continued in these laboratories on the basis of the above hypothesis.

References and Notes

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of the Meeting," vol. 8, Academic Press, London, 1973, p95.

10) F. Yoneda, Y. Sakuma, and P. Hemmerich, *J. Chem. Soc., Chem. Commun.*, 1977, 825.

11) A. Shirra and C.J. Suckling, *J. Chem. Soc., Perkin Trans.* II, 1977, 759.

12) In the presence of *N*-substituted nicotinamides and excess KOH, salicyl alcohol was converted to salicylaldehyde which was easily detected by NMR. However, the corresponding dihydronicotinamides were neither detected by NMR nor isolated by solvent extraction. The NMR peak of the aldehyde group was also observed for the reaction mixture carried out in the absence of nicotinamides. Conceivably, salicyl alcohol was *air-oxidized* under the alkaline conditions. A similar observation was communicated personally by Dr. Y. Ohnishi(Sagami Chemical Research Center).

13) For a comprehensive review, see U. Eisner and J. Kuthan, *Chem. Rev.*, 72, 1(1972).

14) The mechanistic evidence for "hydride" transfer is not offered at present. For the sake of simplicity, we adopted the term for the oxidation of alcohols by NAD^+ analogues.

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17) S. Shinakai, K. Tamaki, and T. Kunitake, *Makromol. Chem.*, 178, 133(1977).

18) S. Shinkai and T. Kumtake *Makromol. Chem.*, 178, 145(1977).

19) S.L. Jonson and K. W. Smith, *Biochemistry*, 15, 553(1976).

20) R.N. Lindquist and E.H. Cordes, *J. Am. Chem. Soc.*, 90, 1269(1968).

21) S. Shinakai and T. Kunitake, *Chem. Lett.*, 1977, 297.