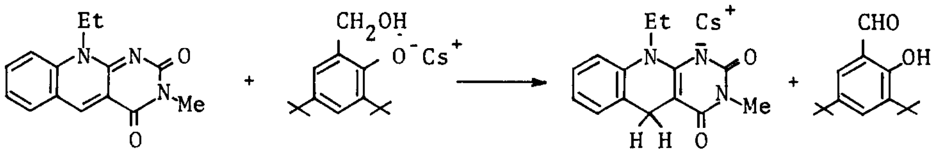
Tetrahedron Letters,Vol.23,No.13,pp 1357-1360,1982 0040-4039/82/131357-04$03.00/0
Printed in Great Britain

SOLVENT EFFECTS IN 5-DEAZAFLAVIN OXIDATION AS A MODEL FOR NAD$^+$ DEPENDENT ENZYMES

Seiji SHINKAI*, Hideo KURODA, and Osamu MANABE
Department of Industrial Chemistry, Faculty of Engineering
Nagasaki University, Nagasaki 852, Japan

The oxidation rate of 3,5-di-t-butylsalicyl alcohol by 3-methyl-10-ethyl-5-deazaisoalloxazine was found to be very sensitive to solvent effect, the rate constant in 99 vol% DMF being greater by a factor of 37 than that in 50.50 (v/v) DMF-water mixed solvent. The result suggests the importance of the aprotic nature of the reaction environment during the oxidation of alcohols by NAD$^+$.

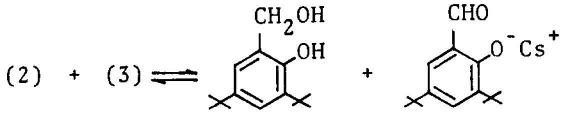
As part of a study to examine the hypothesis that some of the rate acceleration caused by an enzyme may be imitated by different solvent effects, several investigations on the dihydronicotinamide reduction as a model for NADH dependent enzymes have been reported. van Eikeren and Greier1) found in the reduction of trifluoroacetophenone by 1-propyl-1,4-dihydronicotinamide in dimethyl sulphoxide-water mixture that the reduction rate dramatically increases with increasing water concentration. We also reported that some of the NADH model reduction processes are subject to general-acid catalysis.2,3) These findings are in line with a well-established fact in the NADH model reduction that the ortho-hydroxyl group is able to facilitate the reduction of various double bonds.4-7) The relevance of these studies to the enzymatic mechanism is supported by recent X-ray crystallographic studies of NADH-dependent enzymes that the protonated imidazole of the histidine residue acts as a general acid source during the reduction process.8,9) Based on the principle of the microscopic reversibility, we predicted that the oxidation of alcohols by NAD$^+$ (or its model compounds) would be general-base catalyzed,2) but there has been no suitable system to examine the hypothesis. Very recently, Yoneda et al.10) demonstrated that 5-deazaflavin (or 5-deazaisoalloxazin which has redox properties similar to NAD$^+$ and is thus called nicotinamide in flavin clothing,11,12) is able to oxidize alcohols quantitatively to corresponding carbonyl compounds. In this communication, we report for the first time that the oxidation of alcohols by 3-methyl-10-ethylisoalloxazine(1) as an NAD$^+$ model is general-base catalyzed and that the reaction rates are remarkably suppressed by protic solvents. In order to suppress the possible adduct formation between (1) and basic components, we employed a sterically-hindered phenolate, 3,5-di-t-butylsalicyl alcohol anion(2) as substrate.



(1)

(1) (2) (3)

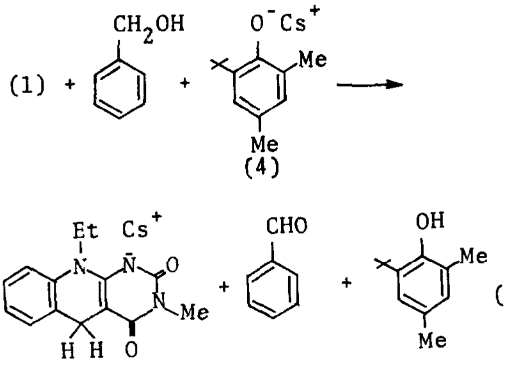
The typical experimental method is as follows. (1) (1.03×10^{-2} mole) and 3,5-di-t-butylsalicyl alcohol (2.06×10^{-2} mole) were dissolved in 10 ml of N,N-dimethylformamide(DMF) (or DMF-water mixed solvent) and the solution was maintained at 50°C. After replacing the atmosphere with nitrogen, the reaction was initiated by adding 100 µl of aqueous CsOH (1.03×10^{-2} mole). The aliquot was withdrawn from the solution at appropriate time intervals and the reaction was stopped by mixing with acetic acid. The yield of 3,5-di-t-butylsalicylaldehyde(3) was determined by a glc method with acetanilide as internal standard. As the reaction proceeds, the equilibrium of Eq. 2 should lead to the shift of medium pH, resulting in more rapid decrease of anionic (2) than that of (1). The analytical method for such pH-shifting reaction system has been reported.[6] We thus determined the second-order rate constants k_2 ($= v_{obsd} / [(1)][(2)]$) from the plots of produced (3) versus reaction time according to the method.



(2)

The results are illustrated in Figure 1. The k_2 in 99 vol% DMF was 0.55 M^{-1} sec^{-1}. The rate constants sharply decreased with increasing water concentration, and the k_2 in 50 50 (v/v) DMF-water mixed solvent was 0.0147 M^{-1} sec^{-1} which is smaller by a factor of 37 than that in 99 vol% DMF. From a viewpoint of solvent effects, the finding can be elucidated on the basis of the work of Parker and others[13] that is, the reactivities of anions are generally quenched in protic solvents owing to the favorable solvation through hydrogen bonding. From a viewpoint of the enzyme model reaction, it leads to a speculation that, contrary to the favorable influence of the protic nature on the reduction process,[1-7] the NAD^+ oxidation of alcohols would be facilitated by the aprotic reaction environment.

The efficiency of the ortho-phenolate group as intramolecular general base in (2) was demonstrated by comparing the rate constant with that for the oxidation of benzyl alcohol (2.06×10^{-2} mole) by (1) (1.03×10^{-2} mole) with the aid of 2,4-dimethyl-6-t-butylphenol (2.06×10^{-2} mole)(4) + CsOH (1.03×10^{-2} mole) as intermolecular general base (Eq. 3).



(3)

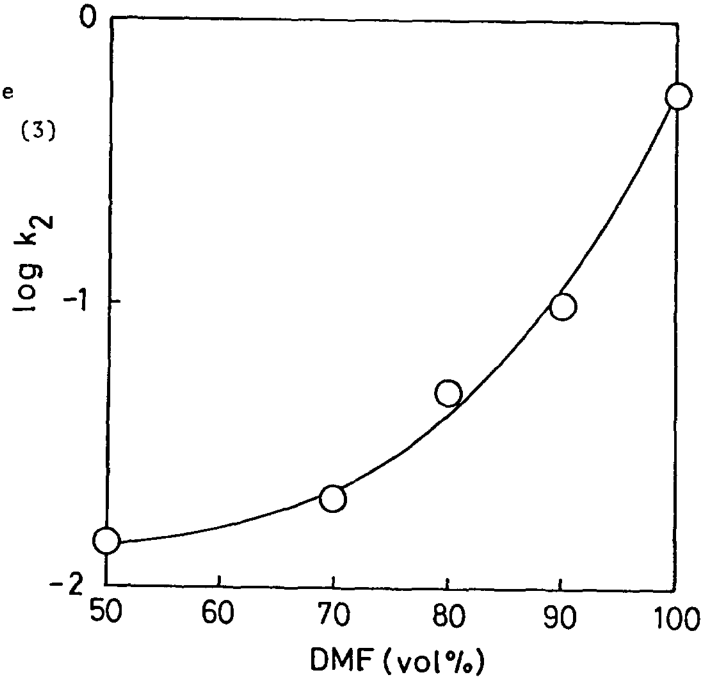


Figure 1. Dependence of log k_2 on the concentration of water present during the oxidation of 3,5-di-t-butylsalicyl alcohol by 3-methyl-10-ethyl-5-deazaisoalloxazine

In 90 vol% DMF solution, the reaction was followed by monitoring the yield of benzaldehyde by a glc method. The reaction was found to be very slow, the yield of benzaldehyde based on (1) after 110 min being only 2.3%. The apparent second-order rate constant k_2 (= v_{obsd} / [(1)][(4)] was 3.5×10^{-4} M^{-1} sec^{-1}, which is smaller by a factor of 286 than that for Eq. 1 in 90 vol% DMF ($k_2 = 0.10$ M^{-1} sec^{-1}).

In conclusion, the oxidation by 5-deazaflavin (and probably also by NAD$^+$) is facilitated by the aprotic reaction environment and by the presence of intramolecular general base. The results support that the principle of the microscopic reversibility is operative in oxidation-reduction reactions coupled with the interconversion of NAD$^+$-NADH.

References

1) P. van Eikeren and D. L. Grier, J. Am. Chem. Soc., 98, 4655 (1976).
2) S. Shinkai and T. Kunitake, Chem. Lett., 1977, 297.
3) S. Shinkai, H. Hamada, T. Ide, and O. Manabe, Chem. Lett., 1978, 685; S. Shinkai, H. Hamada, Y. Kusano, and O. Manabe, J.C.S. Perkin II, 1979, 699.
4) R. H. Abeles, R. F. Hutton, and F. H. Westheimer, J. Am. Chem. Soc., 79, 712 (1957).

5) U. K. Pandit and F. R. Mas Cabre, *J.C.S. Chem. Comm.*, **1971**, 552.

6) S. Shinkai and T. C. Bruice, *Biochemistry*, **12**, 1750 (1973).

7) S. Shinkai, S. Shiraishi, and T. Kunitake, *Bull. Chem. Soc. Japan*, **49**, 3656 (1976) S. Shinkai, Y. Kusano, T. Ide, T. Sone, and O. Manabe, *ibid.*, **51**, 3544 (1978).

8) M. J. Adams, M. Buehner, K. Chandrasekhar, G. C. Ford, M. L. Hackert, A. Liljas, M. G. Rossman, I. E. Smiley, W. S. Allison, J. Everse, N. O. Kaplan, and S. S. Taylor, *Proc. Nat. Acad. Sci. USA*, **70**, 1968 (1973).

9) D. Moras, K. W. Olsen, M. N. Sebesan, M. Buehner, G. C. Ford, and M. G. Rossmann, *J. Biol. Chem.*, **250**, 9137 (1975).

10) F. Yoneda, Y. Sakuma, and P. Hemmerich, *J.C.S. Chem. Comm.*, **1977**, 825.

11) C. Walsh, *Acc. Chem. Res.*, **13**, 148 (1980).

12) P. Hemmerich, V. Massey, and H. Fenner, *FEBS Lett.*, **84**, 5 (1977).

13) A. J. Parker, *Chem. Rev.*, **69**, 1 (1969) and references cited therein.

(Received in Japan 30 December 1981)