Micellar Catalysis of Oxidation of Nitroethane by Isoalloxazine (Flavin)

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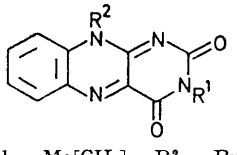
Summary Oxidation of nitroethane by flavins to acetaldehyde and nitrite ion, usually not possible in nonenzymatic systems, does take place with an isoalloxazine (flavin) bound to a cationic micelle.

PORTER and his co-workers[1] have found that D-amino acid oxidase (flavoenzyme) rapidly oxidizes nitroalkanes to the corresponding aldehydes and nitrite ion, and that the enzyme employs the flavin 5-adduct with the nitroalkane as the intermediate which can be irreversibly trapped by cyanide ions. In nonenzymatic systems, this reaction does not proceed, unless a very electron-deficient isoalloxazine, 3,10-dimethyl-8-cyanoisoalloxazine is employed.[2] We have found that an isoalloxazine bound to a cationic micelle does catalyse the oxidation of nitroethane.

3-Hexadecyl-10-butylisoalloxazine (**1**), m.p. 77—80 °C, was prepared from hexadecyl iodide and 10-butylisoalloxazine[3] in the presence of K_2CO_3, and identified by n.m.r. and elemental analysis. The reactions of isoalloxazines with nitroethane were performed at 30 °C, pH 8—9, under anaerobic (N_2) conditions.



(**1**) $R^1 = Me[CH_2]_{15}$, $R^2 = Bu^n$
(**2**) $R^1 = Me$, $R^2 = Et$
(**3**) $R^1 = Me$, $R^2 = Bu^n$

3-Methyl-10-ethylisoalloxazine (**2**)[3] and 3-methyl-10-butylisoalloxazine (**3**)[3] did not react with nitroethane in a non-micellar system. Instead, slow hydrolysis was observed. Addition of hexadecyltrimethylammonium bro-

mide (CTAB: 3 mM)† to these systems only accelerated the hydrolysis rate (2—3 fold). However, reduction and hydrolysis of (**1**) occurred competitively in the presence of the cationic micelle CTAB (3 mM); the lower the pH of the medium, the more reduced isoalloxazine was produced (*e.g.*, 100% at pH 8·06; 60% at pH 8·73).‡ Since (**1**) is not very soluble by itself in aqueous solutions, it is suggested that the isoalloxazine can be reduced by nitroethane when solubilized in the micellar phase (*vide infra*).

Kinetic measurements for the reaction of (**1**) with nitroethane in the micellar system§ were carried out under pseudo-first-order condition (excess of nitroethane), and the rate constants (k_1') were determined ($k_1' = k_{obs} - k_{hydrolysis}$). The reaction was found to be first-order in (**1**) and nitroethane, and the pH dependence of the apparent second-order rate constants (k_2') was precisely reproduced by the quantity $k_2K_a/(K_a + a_H)$ with K_a (acid dissociation constant) = $2{\cdot}0 \times 10^{-9}$ M and k_2 (second-order rate constant for oxidation of the nitroethane anion) = $3{\cdot}63 \times 10^{-4}$ l mol^{-1} s^{-1}. The value of K_a kinetically determined in the micellar system can be compared to that in a nonmicellar system ($2{\cdot}5 \times 10^{-9}$ M at 25 °C),[4] so that the acidity of nitroethane is apparently unaffected in the micellar system. Thus, the reaction rate is given by equation (1). These

$$-\mathrm{d}[(\mathbf{1})]/\mathrm{d}t = k_2\,[(\mathbf{1})]\,[\mathrm{Me}\bar{\mathrm{C}}\mathrm{HNO_2}] \qquad (1)$$

results prove that the cationic micellar environment can catalyse the flavin-mediated oxidation of nitroethane anion to acetaldehyde and (probably) nitrite ion.¶ Since the oxidation of nitroethane virtually did not occur in a nonmicellar system ($k_2 < 10^{-7}$ l mol^{-1} s^{-1}), the rate increase observed amounts to more than three orders of magnitude.

The spectra of (**2**) and (**3**) in the micellar system were similar to those in bulk water. In contrast, the visible spectrum of (**1**) in the presence of the cationic micelle (λ_{max} 440 nm) possessed a shoulder at 460—470 nm which is characteristic of flavins in organic solvents (MeCN, dioxan). Therefore, the isoalloxazine ring of (**1**) must be present in the hydrophobic region of the micelle. Thus, the reaction may be facilitated by (i) the micellar environment favourable for the reduction of flavins as in some dipolar aprotic solvents[5] and/or (ii) the activation of the nitroethane anion due to the formation of 'hydrophobic ion pairs.'[6,7] In the latter, the anion included in a hydrophobic environment will attain its high reactivity from desolvation (dehydration).

In conclusion, the present study suggests the importance of hydrophobic environments in flavin-mediated oxidation–reduction reactions. Since the coenzyme binding site of apoenzymes is said to be situated in relatively hydrophobic environments, micellar catalysis may have an important bearing upon microenvironmental effects in enzymatic reactions.

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† The critical micellar concentration under the kinetic conditions is $8{\cdot}0 \times 10^{-4}$ M (ref. 7).

‡ The amount of the reduced fraction was estimated by the absorbance increase at 440 nm [λ_{max} of the isoalloxazine (**1**)] after reoxidation with O_2.

§ 30 °C, pH 8·0—9·2, 0·1 M borate buffer, μ 0·15 M with KCl, 3 vol% ethanol, [CTAB] $3{\cdot}0 \times 10^{-3}$ M.

¶ Carried out on a preparative scale; acetaldehyde was identified by distillation from acidified reaction mixture and isolation as its semicarbazone derivative: m.p. 160—162 °C.

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