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ON THE PECULIAR REACTIVITIES OF N(5)-HYDROGEN-BONDED FLAVIN. A MODEL APPROACH TO FLAVIN ACTIVATION THROUGH HYDROGEN-BONDING

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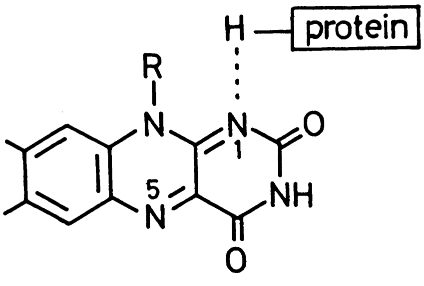
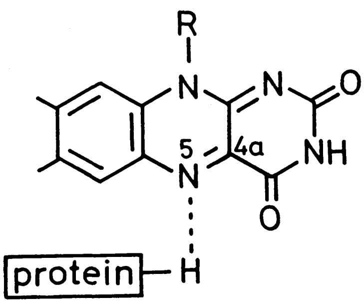
A new flavin with hydrogen-bonded N(5) (OHFl: sodium 1'-hydroxybenzo[2', 3'-h]-10-methylisoalloxazine-5'-sulfate) was synthesized. The reactivity for NADH model compounds was similar to that of 3-methyllumiflavin(MeLFl), whereas the rate constants for the reactions with thiols were enhanced by 33-645 fold. The result implies that OHFl is "specifically" active towards thiol oxidation, the reaction involving 4a-intermediates. Thus, OHFl can mimick part of the hydrogen-bond-dependent activation mechanism of flavoproteins.

Flavin coenzymes are versatile redox catalysts in biological systems and it is now known that more than 100 proteins require flavin coenzymes. Recently, Massey and Hemmerich$^{1)}$ suggested that these flavoproteins may be classified into two classes from a viewpoint of their reactivities: the first group which is represented by dehydrogenases/oxidases features red semiquinone radical, high sulfite affinity, and bent structure of reduced form, whereas the second group which is represented by electron-transferases(or dehydrogenases/electron-transferases) features blue semiquinone radical, low sulfite affinity, and almost planar structure of reduced form. They proposed that the essential difference stems from the position of hydrogen-bonding with proteins: the former has the hydrogen-bond with N(1) (1) leading to the activation of N(5), while the latter has the hydrogen-bond with N(5) (2) leading to the activation of 4a-position. The situation reminds us of the role of the 3-hydroxyl group in pyridoxal coenzymes activating the neighboring aldehyde group.

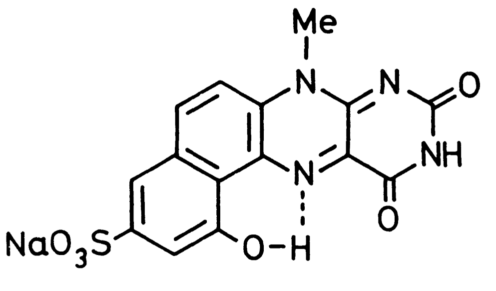


(1)

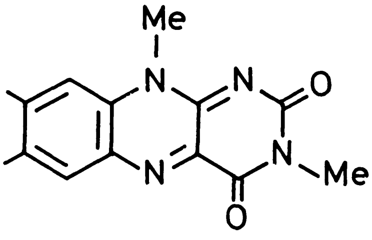


(2)

In order to obtain an insight into the role of hydrogen-bonding, we synthesized a new flavin with hydrogen-bonded N(5) (OHFl: sodium 1'-hydroxybenzo[2', 3'-h]-10-methylisoalloxazine-5'-sulfate). OHFl contains within a molecule both isoalloxazine and proton-sponge(1,8-bis(dimethylamino)naphthalene)-like structures. We have found that the reactivities of OHFl are remarkably different from those of 3-methyllumiflavin(MeLFl) used as a reference flavin.

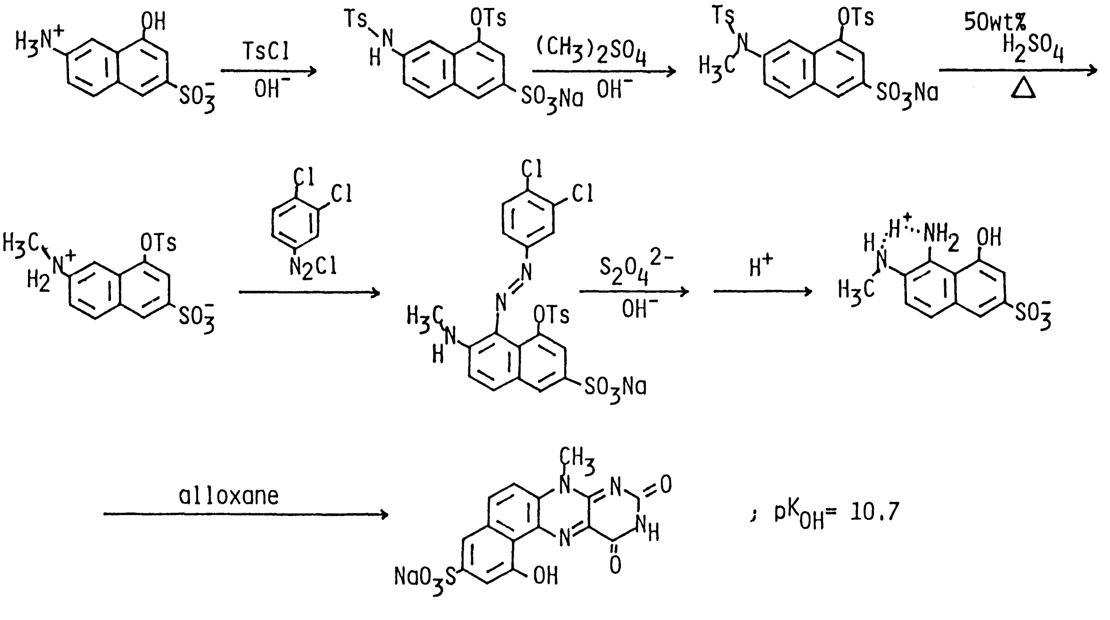


OHFl



MeLFl

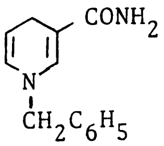
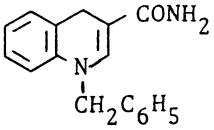
OHFl was synthesized from γ-acid according to the following reaction scheme and identified by IR, NMR, and elemental analysis: λ_{max}(water) 497 nm; IR(KBr) ν_{OH} 3440 cm^{-1}, $\nu_{C=O}$ 1714, 1665 cm^{-1}; NMR(Me_2SO-d_6) δ 4.07(CH_3, 3H), 7.33(4'-H or 6'-H, 1H), 7.79(6'-H or 4'-H, 1H), 7.89(9-H, 1H), 8.38(8-H, 1H), 11.49(NH or OH, 1H), 12.69(OH or NH, 1H).



First, we examined the reaction with NADH model compounds, 1-benzyl-1,4-dihydronicotinamide(BzlNAH) and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline(BzlQH)[2] at 30 °C. The reaction is a typical inter-coenzyme reaction and first-order in flavin and NADH model compounds. According to Gascoigne and Radda,[3] the logarithm of the second-order rate constants(k_2) is linearly correlated with the polarographic half-wave potentials of flavins. Table 1 shows that the rate constants for

OHFl are quite comparable with those of MeLFl. The result indicates that there is little difference in the redox potentials between OHFl and MeLFl.

Table 1. Rate constants for the reactions with OHFl and MeLFl (30 °C)

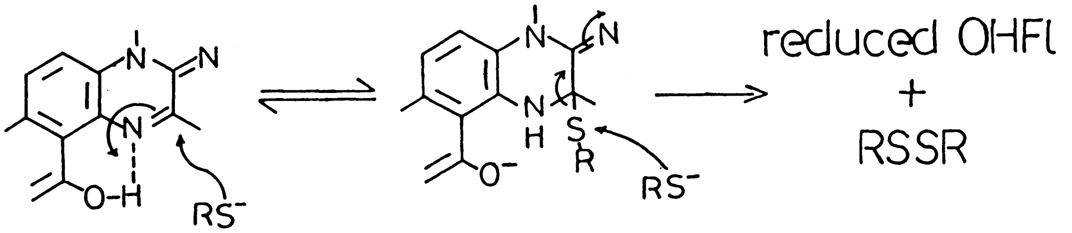
Reactant	Conditions	Rate constant		
			OHFl	MeLFl
	pH 8.81 with 0.010 M carbonate	$k_2/M^{-1}s^{-1}$	11.1	12.1
	pH 5.69 with 0.010 M acetate	$k_2/M^{-1}s^{-1}$	0.402	0.311
$HS(CH_2)_4SH$[a]	pH 9.80 with 0.10 M carbonate (20 vol% EtOH)	k_1'/s^{-1}	7.10×10^{-3}	2.13×10^{-4}
$HO(CH_2)_2SH$[b]	pH 9.20 with 0.33 M carbonate	k_1'/s^{-1}	4.43×10^{-3}	6.87×10^{-6}
K_2SO_3[c]	pH 7.21 with 0.50 M phosphate	k_1'/s^{-1}	3.05×10^{-2}	1.67×10^{-5}

[a] [1,4-butanedithiol]=2.40×10^{-3} M.

[b] [2-mercaptoethanol]=0.0180 M.

[c] $[SO_3^{2-}] + [HSO_3^{-}] = 1.56 \times 10^{-3}$ M.

It is known that flavin oxidation of mono- and dithiols proceeds via the formation of 4a-adducts with first thiolate followed by the breakdown with second thiolate.[4] We found that, being different from the reaction with NADH model compounds, the pseudo-first-order rate constants (k_1') for OHFl are greater by 33-645 fold than those for MeLFl(Table 1). The result implies that OHFl is "specifically" active towards the reactions involving 4a-intermediates. This is readily ascribed to 4a-activation through hydrogen-bonding with N(5).



(1)

3,10-Dimethyl-7-cyanoisoalloxazine(CNFl), which has a high activity due to the electron-withdrawing cyano group, rapidly oxidized BzlNAH, the rate constant being greater by 58-fold than that for OHFl. In contrast, the rate constants for oxidation of 2-mercaptoethanol were quite similar ($k_{CNFl}/k_{OHFl}=1.4$). The result again supports the "specific" reactivity of OHFl.

We found that the reaction of OHFl with SO_3^{2-} is further enhanced (1830 fold!) relative to that of MeLFl. Further interesting is the fact that the OHFl-SO_3^{2-} adduct (λ_{max} 458 nm) slowly decays to 1,5-dihydro-OHFl. It is known that the nucleophilic attack of SO_3^{2-} usually occurs at N(5) while the attack at 4a becomes favorable in the presence of HSO_3^- acting as general acid on N(5),[5] and 1,5-dihydroflavin results only from 4a-adduct as shown in Eq. 1. According to the Corey-Pauling-Koltun(CPK) model, N(5) of OHFl seems sterically protected from the nucleophilic attack of SO_3^{2-}. At present, we presume that with the aid of intramolecular general acid on N(5), the reaction between OHFl and SO_3^{2-} forms the 4a-adduct which is subsequently converted to 1,5-dihydro-OHFl.

In conclusion, the present paper demonstrates that OHFl well mimicks the activation mechanism of flavoproteins containing N(5)-hydrogen-bonded flavin coenzymes. To obtain further insight into the flavin activation through hydrogen-bonding, we are now synthesizing an N(1)-hydrogen-bonded flavin model compound which would act as a model for the another activation mechanism.

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References

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