A Model for Controlling Redox Reactivities of Flavins by Conformational Changes between Oxidized and Reduced States

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The 9,10-ethylene linked flavin (**1**) is more reactive than the 9,10-trimethylene linked flavin (**2**) for oxidation ($F_{ox.} \rightarrow FH_2$), and less reactive to reduction [$FH_2 \rightarrow F_{ox.}$ and $FH_2 \rightarrow$ ·FM (metal-chelated flavin radical)], evidence that ·FM contains an almost planar flavin moiety.

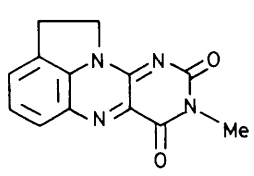
Oxidized flavins ($F_{ox.}$) are planar molecules, while reduced flavins are known to have a bent structure which resembles a butterfly with an N(5)–N(10) axis.[1] This suggests that enforced bending of the $F_{ox.}$ molecule increases its oxidizing power and enforced flattening of the FH_2 molecule increases its reducing power. In this sense, a flavin could be regarded as a unique molecule that changes conformation on losing and gaining electrons.

This possibility was examined kinetically by employing strained flavins (**1**) and (**2**) in (a) the oxidation of *N*-benzyl-1,4-dihydronicotinamide (BNAH), (b) the reduction of 2,4-dinitrochlorobenzene, and (c) the one electron reduction of *p*-chloronitrobenzene in the presence of Ni^{2+} [examples of $F_{ox.} \rightarrow FH_2$, $FH_2 \rightarrow F_{ox.}$, and $FH_2 \rightarrow$ ·FM (M = metal), respectively].

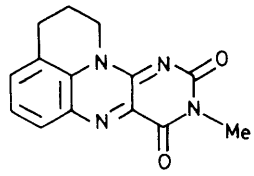
Flavins (**1**) and (**2**) were prepared from indoline or 1,2,3,4-tetrahydroquinoline and 6-chloro-3-methyluracil according to the procedures of Yoneda.[2] Analytical data (u.v.–visible and elemental analysis) were satisfactory. Corey–Pauling–Koltun (CPK) molecular models of (**1**) and (**2**) suggest that they are more strained as $F_{ox.}$ rather than FH_2, and the strain released by changing from $F_{ox.}$ to FH_2 is larger for (**1**) than for (**2**).

$$F_{ox.} \rightarrow FH_2 \quad e.g.\ (a)$$
$$FH_2 \rightarrow F_{ox.} \quad e.g.\ (b)$$
$$FH_2 \rightarrow \cdot FM \quad e.g.\ (c)$$

$$(FH^- + M^{2+} \overset{K}{\rightleftharpoons} FH^-M^{2+} \xrightarrow{k_2[S]} \cdot FM + \text{product})$$



(**1**)



(**2**)

The rate constants were determined spectrophotometrically by following the decrease in absorption due to $F_{ox.}$ at 440 nm for reaction (a), the increase for reaction (b), and the increase in absorption (371 nm)[3] due to the metal-chelated flavin radical for reaction (c) under anaerobic conditions. All the reactions followed good first-order kinetics up to more than two half lives. The results are summarized in Table 1. The rate difference between (**1**) and (**2**) in Table 1 may be accounted for by strain changes involved in the flavins owing to the conformation changes, since electronic effects of ethylene and

Table 1. Rate constants at 25 °C.

Reactions	$k_{obs.}$/min^{-1}		Relative rate [(2)/(1)]
	(1)	(2)	
(a)[a]	0.800	0.417	0.52
(b)[b]	0.260	1.10	4.2
(c)[c,d]	4.26×10^3	8.33×10^3	2.0

[a] [$F_{ox.}$] 5×10^{-5} M, [BNAH] 1×10^{-3} M, pH 8.11 (0.1 M phosphate, μ 0.3). [b] [FH_2] 5×10^{-5} M, [2,4-dinitrochlorobenzene] 3×10^{-3} M in EtOH containing *N*-ethylmorpholine (1×10^{-3} M) as a photoreductant. [c] [FH_2] 5×10^{-5} M, [*p*-chloronitrobenzene] 1×10^{-3} M in EtOH containing *N*-ethylmorpholine (1×10^{-3} M) in the presence of Ni^{2+}. [d] These values (k_2/dm^3 mol^{-1} min^{-1}) were obtained by varying Ni^{2+} concentrations according to the scheme shown in parentheses after equation (c) (ref. 3).

trimethylene groups linked at the 9,10-positions of isoalloxazine rings are considered to be almost the same. Compound (**1**) is more reactive than (**2**) for $F_{ox.} \rightarrow FH_2$ [reaction (a)], and less reactive for $FH_2 \rightarrow F_{ox.}$ [reaction (b)]; clearly the strain in a flavin molecule affects its oxidation–reduction reactivity.

To date conformations of metal-free and -chelated flavin radicals have not been established in model systems, although an air-stable flavodoxin semiquinone has been proposed to be planar rather than bent.[4] In reaction (c) in which metal-chelated flavin radicals are formed, (**2**) was found to be more reactive than (**1**) [as observed in reaction (b)], and a large rate acceleration due to the metal ion was observed. A higher reactivity of (**2**) compared with (**1**) suggests that the metal-chelated flavin radical is closer to being planar than bent. The results also support our previous proposal that rate acceleration by metal ions is caused by their co-ordination to C(4)=O and N(5) of FH_2 to produce strain by enforced flattening, for which the lone electron pair on N(5) must be equatorial.[3] If the lone electron pair on N(5) takes an axial position, the metal ion becomes chelated by a strong bidentate ligand [N(5) and N(10)] to cause rate retardation, since lone electron pairs on N(10) are compelled to occupy axial positions for (**1**) and (**2**).

The introduction of strain or distortion in a substrate and/or a protein is thought to be, in some cases, one of the factors bringing about rate acceleration in enzyme catalysis.[5] The present study may allow us to say that a metal-chelated flavin radical is a planar rather than a bent molecule, and oxidation–reduction reactivity of a flavin coenzyme is affected by conformational change caused by the apoenzyme tightly binding the coenzyme.

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