5-(2-THIOLYL)-1,5-DIHYDRO-FLAVIN: A MODEL OF GROUP TRANSFER IN FLAVIN DEPENDENT SUBSTRATE DEHYDROGENATION

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1. Introduction

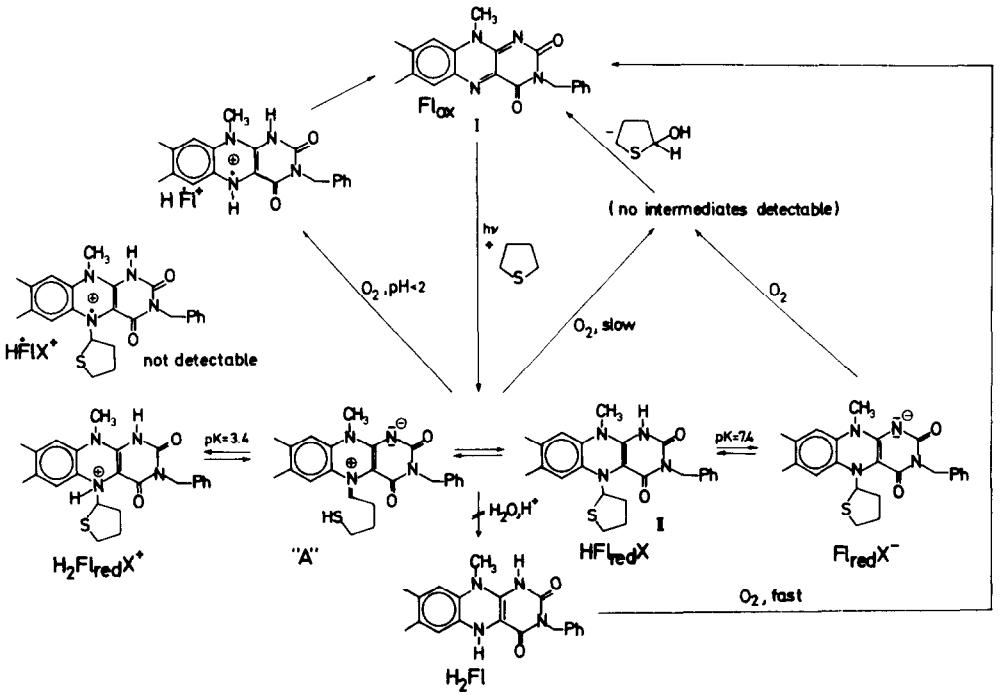
In a recent review [1] we have collected data in support of the idea that flavin dependent substrate dehydrogenation does not involve 'hydride' transfer, as generally believed (i.e. substrate being split by flavin as $XH + Fl_{ox} \rightarrow Fl_{red}H^- + X^+$) but instead "group" transfer ($XH + Fl_{ox} \rightarrow Fl_{red}X^- + H^+$). Such an idea is rationalized in two ways: First, the reduction of flavoprotein dehydrogenases by their substrates yields, in general, neither fully reduced 1,5-dihydroflavin $Fl_{red}H_2$ nor flavosemiquinone FlH as first intermediates, but flavin-substrate adducts [2, 3] which, because of their red color, are generally considered as 'π-complexes'. But it is hard to visualize what kind of π-bonding should originate from substrates like, e.g., alanine [2, 3]. Hence, it is much more satisfying to assume 'σ-complexes' $Fl_{red}X^-$, whose red color is of course still unexplained in terms of the molecular structure. Second, studies of (photo)chemical 'substrate' dehydrogenation [4] reveal that quantitative formation of free flavohydroquinone $Fl_{red}H_2$, as achieved with EDTA as 'substrate', is rare. We call this the 'reversible' photoreduction since $Fl_{red}H_2$ is autoxidized instantaneously. With an increasingly large number of other photosubstrates, flavin reduction is partially (e.g. pyruvate as substrate [5]), if not entirely (phenylacetate as substrate [6, 7]), irreversible because of $HFl_{red}X$ formation. Reoxidation of the 'adducts' $Fl_{red}XH$ is accompanied by removal of X and is, therefore, usually slow and thermodynamically irreversible.

For a 'biological model' reaction, those adducts $Fl_{red}XH$ which are split by O_2 in the dark are most interesting. Furthermore, the question arises: at which redox state (reduced, radical or oxidized = hydroquinone, semiquinone or quinone state) the Fl–X rupture takes place, and whether water is involved in the reaction ($X^+ + H_2O \longrightarrow XOH + H^+$).

We wish to report the synthesis of an analogous adduct with a substituent X, which arises from dehydrogenation of XH rather than decarboxylation of XCO_2H, as in all the above cases. Furthermore, the new X is fixed to flavin-N(5) by a carbon atom bearing a second functional group. This renders X more similar to the natural substrate residues from e.g. α-amino acids ($X = RC(NH_2)CO_2^-$). Hence, it is questionable whether even in the 'reversible' flavin-dependent dehydrogenation the apparent 'hydride transfer' is not *simulated* by a sequence of rate limiting group transfer and rapid hydrolysis.

2. Results and discussion

Photoreduction of flavin derivatives (Fl_{ox}) occurs rapidly in tungsten light with thiolane (tetrahydrothiophene) as substrate. The residue $X = C_4H_7S$ is trapped with a yield of $> 90\%$ at the flavin nucleus in position 5, yielding an intermediate $HFl_{red}X$, $\lambda_{max} = 335$ nm, of composition $C_{24}H_{26}N_4O_2S$, (where Fl_{ox} = 3-benzyl-lumiflavin $C_{20}H_{18}N_4O_2$) sufficiently stable for isolation (cf. scheme 1). The structure is derived from the ^{1}H-NMR spectrum which shows a CH-triplet with $\delta = 5.30$ ppm and $J = 6.5$ Hz indicative of a $\begin{matrix} -S \\ -CH \end{matrix}\!\!>\!CH-N(5)$-linkage. This species exhibits two pK_a values, one at 7.4 arising from deprotonation of N(1) to yield $Fl_{red}X^-$, as is normal for flavohydroquinones [7, 8], and one at 3.4 due to



Scheme 1. Formation and oxidative decay of 3-benzyl-5-(2-thiolyl)-1,5-dihydro-flavin (II).

protonation of N(5) ($H_2Fl_{red}X^+$, λ_{max} = 310 nm). Fig. 1 shows the spectral course of its formation and decay by O_2. The pH-dependence of this reoxidation is shown in fig. 2. The seemingly complex pH-function reflects a general acid catalysis (rate increase from pH 10 ⟶ 8 and from pH 4 ⟶ 3.5) interrupted by protonation of the flavin nucleus first at N(1), pK = 7.4 and then at N(5), pK = 3.4. The general acid catalysis should therefore be due to protonation not in the nucleus but in the 5-substituent, i.e. at the sulphur. The assumption of the equilibrium $HFl_{red}X \rightleftarrows$ 'A' (scheme 1) then follows from the fact that anaerobic preincubation of $HFl_{red}X$ at acidic pH does not alter the UV-spectrum nor increase the oxidation rate, as should be the case if $HFl_{red}X$ were hydrolyzed irreversibly to yield the instantaneously autoxidizable H_2Fl_{red} (fig. 1). Hence, it must be assumed that an intermediate 'A' (cf. scheme 1) is attacked rapidly by O_2. This is understandable, since 'A' is planar in contrast to $HFl_{red}X$. Quite generally 1,5-dihydro-flavins are bent [10, 11] and the ease of autoxidation seems to be proportional to the inversion rate of the bent state [12], i.e. in as much as the inversion of the 'butterfly wing' conformers is slowed down, the autoxidation rate decreases. II should exhibit a slower inversion of the N(5, 10) center because of the bulkiness of the 5-substituent.

No stable radical is formed upon reaction of $HFl_{red}X$ with O_2, unlike the case of saturated alkyl substituents at N(5), where stable neutral radicals $\dot{F}lX$ are obtained [13, 14]. Instead, oxidation of $HFl_{red}X$ by O_2, $K_3[Fe(CN)_6]$ or Ce^{4+} leads to formation of Fl_{ox} in quantitative yield, while the thiolyl residue is split off as 2-hydroxy-thiolane (which equilibrates in water rapidly to its open tautomeric form, 4-mercapto-butyraldehyde [15]). The instability of $\dot{F}lX$ with X = C_4H_7S is likely to be due to the fact that this bulky residue cannot easily be accommodated in the flavin plane (as required for a stable radical) and is furthermore readily cleaved by solvolysis unlike simple alkyl residues. Under acidic conditions, the corresponding radical cation $H\dot{F}lX^+$ is also unstable and is hydrolyzed instantaneously to yield $H_2\dot{F}l^+$ + XOH. This is evident from the ESR-spectrum, which

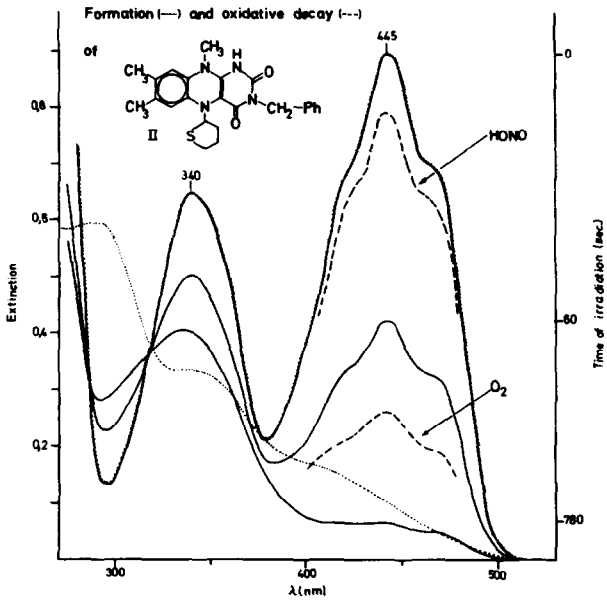


Fig. 1. Flavin-dependent photodehydrogenation of II (CH_3CN, 0.73×10^{-4} M 3-benzyl-lumiflavin [9], 0.067 M thiolane strictly anaerobic conditions, 250 W/24 V tungsten-halogen-lamp with a filter and lens system transparent from 300 to 800 nm).
(——): Spectrum of the reaction mixture after 0,60 and 780 sec of irradiation.
(- - - -): Spectrum after reoxidation with air (10 min) and HONO respectively.
(. . . .): Spectrum of photolytically generated 1,5-dihydroflavin (1,4-cyclohexadiene as substrate) and after 3 min aeration, resp.

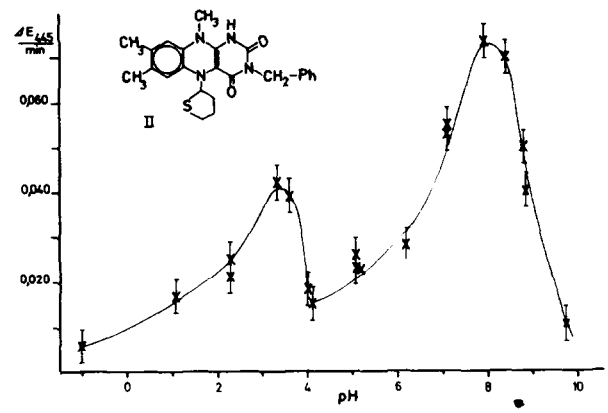


Fig. 2. pH-Dependence of the air-oxidation of II, as measured by the rate of increase of absorption at 445 nm due to formation of Fl_{ox}. Saturated solution of II in methanol (7×10^{-4} M, diluted with an eight-fold volume of 0.1 M aqueous anionic buffers.

shows hyperfine structure from one exchangeable proton (CF_3CO_2H/CF_3CO_2D).

We consider the species $HFl_{red}X$ (scheme 1) as a potential model for a flavoprotein substrate complex for the following reasons:

1) The flavin radical $\dot{F}lX$ proves to be unstable towards solvolytic rupture of the Fl-X bond because of steric hindrance of planarity at N(5). $HFl_{red}X$, therefore, must arise from the flavoquinone triplet by simultaneous two-centered addition of HX, which means a two-electron transfer. Flavin-dependent biological dehydrogenations are known to be two-electron processes [1] which do not yield catalytically essential amounts of radicals.

2) The intermediate $HFl_{red}X$ in its isomeric mesoionic form 'A' would provide an explanation of the color of the biologically observed flavin-substrate complexes.

3) The reoxidation of $HFl_{red}X$ with O_2 is slow, though feasible under physiological conditions, while it is fast with one-electron acceptors such as ferricyanide. This behaviour is characteristic of flavoprotein dehydrogenases [18]. The autoxidation rate is limited by the formation of the isomeric form 'A', which reacts rapidly with O_2, a behaviour characteristic for flavoprotein oxidases [18].

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