By sulfur substitution of the N-5 atom in flavins and flavocoenzymes a flavin analog is obtained, 5-thiaflavin, which is found to be isoelectronic and isosteric with natural flavin in the fully reduced and half-reduced states, but not in the oxidized state. Among the three 'redox shuttles' characterizing the flavin system, viz. upper 1e\textsuperscript{−}, lower 1e\textsuperscript{−} and 2e\textsuperscript{−} shuttle, only the second one is retained in thiaflavin, which limits the redox activity of this system to 1e\textsuperscript{−} transfer.

The structure and properties of the molecular species participating in the thiaflavin redox system are discussed in comparison with the flavin system. The corresponding chemistry of a '2e\textsuperscript{−} flavin', 5-deazaflavin, has been treated in the preceding paper.

5-Thiaflavin is found to exhibit a stable neutral radical, which is analogous to the 'blue' flavosemiquinone. Unlike normal flavin, where the radical is in a dismutation equilibrium, thiaflavin radical shows reversible formation of a covalent dimer, which is stable in aprotic solution and disproportionates only in water, with irreversible formation of a sulfoxide. The ultraviolet and infrared spectra of the dimer are in agreement with the structure of two 5-thiaflavin molecules linked covalently at their 4a carbons. This corroborates the earlier hypothesis that the essential intermediate in the dismutation of normal flavin is likewise a covalent dimer.

Thiaflavin is tightly bound by apoflavodoxin. The protein catalyses the autoxidation to the radical state. Thiaflavodoxin radical is even more stable (towards further oxidation) than is the free thiaflavin radical.

The redox potential of the couple reduced thiaflavin/thiaflavin radical (sFl\textsubscript{red}/sFl\textsuperscript{−}) is surprisingly high. From the reversible equilibrium established with ferricyanide, sFl\textsubscript{red} + Fe(CN)\textsubscript{6}\textsuperscript{3−} ⇔ sFl\textsuperscript{−} + Fe(CN)\textsubscript{6}\textsuperscript{4−}, the standard potential of the sFl\textsubscript{red}/sFl\textsuperscript{−} couple, $E_m$ at pH 7, has been estimated as +0.38 V.

Recently, a modified type of flavocoenzyme, i.e. 5-deazaflavin [1–6], has been the subject of a steadily growing interest. In reviewing the available data on 5-deazaflavoproteins [7], we have reiterated the principles characterizing flavin-dependent redox catalysis, which were established earlier [8].

In contrast to the mandatory 2e\textsuperscript{−} transfer agent nicotinamide, natural flavin appears to be ambiguous by its capability to catalyze 1e\textsuperscript{−} as well as 2e\textsuperscript{−} transfer.

In addition to 5-deazaflavin, which is a flavin analog lacking 1e\textsuperscript{−}-transfer activity [1], we have searched for a modification of the flavin nucleus which would remove the 2e\textsuperscript{−}-transfer activity in favor of 1e\textsuperscript{−} transfer. Such a derivative has been found by replacing the N-5 atom of reduced flavocoenzymes by sulfur. We have reported structures and properties of the thus-formed '5-thiadihydropyflavin' [9–12]. In the present paper we want to show that they retain only the 1e\textsuperscript{−}-transfer activities of the natural flavins. In accord with this concept, sFMN (the 5-thiaflavin analog of FMN) binds to apoflavodoxins forming redox-active stable holoprotein radicals.

RESULTS AND DISCUSSION

The structure and stability of molecular species making up the thiaflavin redox system is outlined in Scheme 1, along with the abbreviations used in this discussion and the absorption maxima, by which the species are characterized.
The species underlined are those which are 'flavin-analogous', i.e. isoelectronic and isochromic with flavin species of the same state of protonation and oxidoreduction. In order to switch back from thiaflavin to natural flavin, the sulfur has to be replaced by NH. From this it is seen that the analogy is confined to the fully reduced and the half-reduced state, while the oxidized states behave differently. This must be compared with the 5-deazaflavin system, where the oxidized state is flavin-analogous, while the reduced and half-reduced states are not.

In the following paragraphs, we want to discuss the thiaflavin system in comparison with the natural flavin system, based upon the information given in Scheme 1. We begin with the reduced state, since the neutral reduced thiaflavin, sFlredH, is the starting material obtained by total synthesis [11] from which all further preparations are derived.

The Reduced Species
sFlred-1,4a-H2, sFlred-1H and sFlred-

In contrast to the isochromic dihydroflavins, all sFlred species are not autoxidizable, unless bound to apoflavodoxin (see below). From their absorption spectra, as shown in Fig 1, the analogy with flavin is obvious for the neutral state and the anion. The end absorption in the visible region is somewhat less pronounced than in flavin which indicates that the free thiaflavin molecule is less planar than dihydroflavin, the anion being even more bent than the neutral sFlredH for reasons of π-electron overcrowding. This enhanced noncoplanarity is also the reason for the lack of O2 reactivity. The sulfide group >S in position 5 is intermediate in reactivity between NH and NCOCH3 [18]. The more planar a dihydroflavin-like species, the more easily autoxidizable [19] it is.

Under strongly acidic conditions, the reduced thiaflavin system deviates from flavin by the bathochromic shift shown in Fig 1 as well as by a CH peak appearing at 5.2 ppm in the NMR spectrum. This clearly indicates C-4a protonation, which is due to the lack of proton affinity inherent to the sulfur as compared to nitrogen. The 4a-R-sFlred-1-H [11] chromophors (λmax = 380 nm) have their analogy in 4a-alkylated dihydroflavin cations 4a-RFl+ (λmax = 385 nm) [20, 21]. This shows that in thiaflavin the reactivity of position 4a is even enhanced in compari-
Fig. 1. Absorption spectra of reduced thiaflavins. $R^2 = H$. The values for the analogs $R^2 = CH_3$ are given in brackets. Solvent: (-----) in methanol; (-----) in 0.1 M NaOH; (-----) in 12 M HCl.

Fig. 2. Absorption spectra of the half-reduced species. (-----) Spectrum 1, sFl in 0.1 M phosphate buffer pH 6.5; spectrum 2, 2 sFl $\rightleftharpoons$ (sFl)$_2$ equilibrium in chloroform; (-----) thiaflavodoxin radical; (-----) HsFl$^+$ in CHCl$_3$/CF$_3$COOH (1/1). For comparison: (-----) sFl$^{an}$ in conc. H$_2$SO$_4$. Note that the neutral sFl disproportionates in water slowly and irreversibly with $t_{1/2} \approx 6$ min, while reacidification of the monomer-dimer equilibrium in CHCl$_3$ leads back to 100% radical cation HsFl$^+$.

son with natural flavin, where previously only alkylation at C-4a [20] but not protonation has been observed.

The Half-Reduced Species
sFl-1-H$^+$, sFl and sFl-4a,4a'-sFl

Upon oxidation under acidic conditions, the very stable dark-red radical cation sFl-1-H$^+$ is produced quantitatively, and can easily be crystallized, in analogy to the flavin radical cations described as early as 1937 by Kuhn and Ströbele [22]. Upon neutralization of the radical cation, the situation becomes more complex.

As indicated in Fig. 2, in neutral aqueous medium, a nearly quantitative amount of neutral, deep-green radical sFl is produced, which decays slowly, but irreversibly by disproportionation with a half time of
Oh*: 5-Thia-5-Deazaflavin, a 1e−-Transferring Flavin Analog

Scheme 2. Dimers of flavin and thiaflavin

about 6 min. Upon reacidification, after 1 h only small amounts of radical cation can be recovered unless concentrated H₂SO₄ is added.

Under aprotic conditions, such as in CHCl₃, however, no irreversible change is observed, although the amount of green neutral radical is far from quantitative, unless very low concentrations of thiaflavin are used. In concentrated solutions pale-yellow crystals can be isolated. When redissolved in CHCl₃, the same equilibrium is obtained as upon neutralisation of the radical cation. Upon reacidification, the radical cation state is restored quantitatively (Fig. 2). From this it is obvious that a reversible dimerisation occurs for which K₂ can be estimated as 43.1 µM instead of irreversible dismutation that prevails in aqueous systems. Notwithstanding the striking similarity between the radical monomers HF₁ and sF₁, the first-mentioned natural flavosemiquinone decays by disproportionation, while the thiaflavin radical forms a σ-covalent colorless dimer. We take this as independent support of the concept, first proposed by Favaudon and Lhoste [23], that the disproportionation of the natural flavosemiquinone also occurs via a σ-covalent dimer as intermediate. (HF₁)₂ is heterolyzed rapidly, while (sF₁)₂ does so only slowly, requiring protic catalysis. We have proposed for (HF₁)₂ the N-5-unprotonated structure 1-H-F₁-8,8'-F₁-H'-1' because of its long-wavelength absorption which we attributed to intramolecular charge transfer (Scheme 2, formula 1). For the pale yellow (sF₁)₂, however, we must postulate the structure sF₁-4a,4a'-sF₁ (Scheme 2, formula 2) since no proton can be dissociated from position 5.

This proposal is based on the agreement with other known 4a derivatives of thiaflavin [11], as documented by the carbonyl stretching range of the infrared spectra shown in Fig. 3. Since substitution at C-4a separates the 4-carbonyl from the chromophore, we must expect a hypsochromic shift along with a decrease in relative intensity in comparison with the 2-carbonyl. This behavior has been verified earlier for flavins substituted in position 4a [20, 25]. It is characteristic that the absorption spectra of the 4a-substituted thiaflavin chromophore depends on the size and the bulkiness of the 4a substituents. Hence, sF₁ox-4a-OCH₃ shows in the first transition λ_max = 329 nm, ε = 8300 M⁻¹ cm⁻¹, while sF₁red-4a-benzyl produces the smaller
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Fig. 4. Low-resolution EPR spectra of thiaflavin radicals. (——) sFl in chloroform; (----) sFl calculated; (-----) HsFl⁺ in CF₃COOH. Deuteration of the environment does not alter these spectra. The spectra are drawn at low resolution and one of them by higher clarity.

Absorption of $\lambda_{\text{max}} = 334$ nm, $\varepsilon = 4600$ M⁻¹ cm⁻¹ [11]. The extremely bulky dimer (sFl)₂ exhibits $\lambda_{\text{max}} = 368$ nm, $\varepsilon = 3800$ M⁻¹ cm⁻¹, because of reduced planarity.

From the concentration dependence of the dimerization equilibrium, the spectrum of pure (sFl)₂ was found to exhibit maxima at 368 and 390(s) nm with absorption coefficients of 3800 and 3200 cm²/mol mono-sFl. This value is comparable with $\lambda_{\text{max}} = 334$ nm, $\varepsilon = 4600$ M⁻¹ cm⁻¹, for sFl_red-4a-benzyl [11]. From it becomes apparent that there is a marked $\pi$-electron interaction between the two halves, which accounts for the ease of heterolytic cleavage under protic conditions. This dismutation reaction is even faster in alcohols than in water.

From the EPR spectra of the cationic and neutral radicals, as shown in Fig. 4, isotope coupling constants are derived for N-10, N-10-CH₃, C-8-CH₃, and C-6-H which are similar to those of the natural flavin radical [26]. Coupling constants were calculated as described in Table 1. The calculation was not applied to the highest state of resolution which is shown in Fig. 5. Table 1 shows that the spin distribution of HFl and sFl is very similar. In particular, the pyrimidine subnucleus (positions 1-4) is nearly devoid of spin density in both cases, while the benzene subnucleus carries an appreciable spin density centered at C-8.

In Table 2 we have compared the absorption properties of HFl and sFl. The values of $\varepsilon$ for sFl are deduced from EPR integration with 3-carbamoyl-2,2,5,5-tetramethyl-pyrrolidin-1-ylxyloxy as standard. The error limit over five measurements was 4.7%. The 2 sFl ⇄ (sFl)₂ equilibrium, as depending on solvent and temperature, is also listed in Table 2.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Coupling constant $a$ for $X = S$</th>
<th>Coupling constant $a$ for $X = NH$ [26]</th>
<th>Coupling constant $a$ for $X = NCH₃$ [26]</th>
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<tr>
<td>N-5</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>H-5s</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>CH₃-5β</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>N-10</td>
<td>0.36</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>H-6x</td>
<td>0.08</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>CH₃-7β</td>
<td>&lt; 0.03</td>
<td>&lt; 0.03</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>CH₃-8β</td>
<td>0.12</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>H-9x</td>
<td>&lt; 0.03</td>
<td>&lt; 0.03</td>
<td>&lt; 0.03</td>
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</tbody>
</table>

The Oxidized Species

$sFl_{\text{ox}}, sFl_{\text{ox}}-4a-X, HsFl_{\text{ox}} \rightarrow O$ and $sFl_{\text{ox}} \rightarrow O^-$

The neutral radical sFl and its dimer disproportionate under aqueous conditions, yielding one half
5-Thia-5-Deazaflavin, a le-Transferring Flavin Analog

Fig. 5. High-resolution EPR spectrum of sFl in CHCl₃

Table 2. Dependence of the thiaflavin radical properties on the environment: temperature, solvent and protein binding

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>λ max</th>
<th>ε (s)Fl</th>
<th>Kd x 10⁷</th>
<th>T</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiaflavodoxin</td>
<td>0.1 M phosphate, pH 7.0</td>
<td>730</td>
<td>3200</td>
<td>≈ 100</td>
<td></td>
<td>288</td>
</tr>
<tr>
<td>sFl</td>
<td>pH 6.5</td>
<td>672</td>
<td>5800ᵇ</td>
<td>100ᵇ</td>
<td>37</td>
<td>431 ± 32ᵉ</td>
</tr>
<tr>
<td>CHCl₃</td>
<td></td>
<td>736</td>
<td>4500 (calc.)</td>
<td>10ᵇ</td>
<td>53</td>
<td>253</td>
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<tr>
<td>CH₃Cl₂</td>
<td></td>
<td>740</td>
<td>24</td>
<td>137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCON(CH₃)₂</td>
<td>740</td>
<td>11</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃CN</td>
<td>740</td>
<td>11</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzene</td>
<td>740</td>
<td>7.8</td>
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<td></td>
</tr>
<tr>
<td>CCl₄</td>
<td>740</td>
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<td>4.5</td>
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<td></td>
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</tr>
<tr>
<td>(C₂H₅)₂O</td>
<td>740</td>
<td>3.1</td>
<td>2.0</td>
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<td></td>
</tr>
<tr>
<td>Flavodoxin</td>
<td>pH 6.0ᵈ</td>
<td>580</td>
<td>4500</td>
<td>≈ 100</td>
<td></td>
<td>277 [32]</td>
</tr>
<tr>
<td>5-Et-F1</td>
<td>pH 5.0, H₂O</td>
<td>580</td>
<td>3600</td>
<td>≈ 100</td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td>EtOH</td>
<td>630</td>
<td>4500</td>
<td>≈ 100</td>
<td></td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>642</td>
<td>4400</td>
<td>≈ 100</td>
<td></td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td>benzene</td>
<td>655</td>
<td>4700</td>
<td>≈ 100</td>
<td></td>
<td></td>
<td>[30]</td>
</tr>
</tbody>
</table>

ᵃ The total thiaflavin concentration was 0.22 mM.
ᵇ These values were extrapolated to zero time.
ᶜ Mean value with standard deviation from 10 measurements.
ᵈ The buffered solution contained 0.15 M sodium acetate and 0.06 M EDTA.

An absorption coefficient of 5800 M⁻¹ cm⁻¹ in protic solvents was obtained by extrapolation to zero time. In the aprotic solvent chloroform it was calculated as 4500 ± 220 M⁻¹ cm⁻¹ (+ 4.7%) by means of double integration of the low-resolution EPR spectrum of sFl and comparison with 3-carbamoyl-2,2,5,5-tetramethyl-pyrrolidin-1-yloxy as a standard. We presume that the absorption coefficient is not changed considerably in the other aprotic solvents. The amount of the (thia)flavin radical relates to a total flavin concentration of 0.1 mM unless indicated otherwise. Kd was determined at ambient temperature, unless another temperature is stated; Kd = [(sFl)²]/[(sFl)₂]. All values were obtained in this work unless indicated otherwise in the last column.

molecule each of starting sFlₚₐ red and sulfoxide HsFlₚₐ → O. The sulfoxide species are characterized by their very intense absorption below 320 nm (cf. Fig.6). If methanol is used instead of water, the oxidized species sFlₚₐ-4a-OCH₃ is obtained (cf. Scheme 1). From this it might be concluded that, in the aqueous system, sFlₚₐ-4a-OH is an intermediate in the sulfoxide formation. This is, however, not true, since treatment of the 4a-methoxy compound with weak aqueous base does not yield sulfoxide anion, but total decay of the tricyclic system through ring opening at the C-4a—S bond. sFlₚₐ-4a-OCH₃ can, on the other hand, be converted into sulfoxide by acid via hydrolysis of the sulfonium cation sFlₚₐ. The latter can be obtained in pure form in concentrated H₂SO₄ solution. sFlₚₐ is more brownish red as compared to the purple radical HsFlₚₐ⁺ (Fig.2), to which it is easily reduced by as weak a reductant as ethanol. It seems that sFlₚₐ in concentrated H₂SO₄ is even slowly reduced by its own methyl substituents. Thus, sFlₚₐ in H₂SO₄ is stable for days
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Fig. 6. Absorption spectra of oxidized thiaflavin species. (---) $sF_{1ax}$-4a-OCH$_3$ in methanol; (- - -) $1H$-$sF_{1ax}$-4a-OCH$_3$ in CF$_3$COOH; (-----) $HsF_{1ax}$-$O$ in methanol; (-----) $sF_{1ax}$-$O$ in 0.1 M NaOH. For the latter spectrum the left-hand absorption scale is valid.

$sF_{1ax}$-4a-OCH$_3$, 4a-Methoxy-3,7,8,10-tetramethyl-2,4-dioxo-2,3,4,5-tetrahydro-pyrimido[5,4-b][1,4]benzothiazine; $HsF_{1ax}$, 3,7,8,10-Tetramethyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimido[5,4-b][1,4]benzothiazine Sulfoxide

if unsubstituted in positions 7 and 8, but is reduced to $HsF_{1ax}^+$ within 4 h when both positions are methylated. Among the oxidized species, only $sF_{1ax}$ is, in principle, flavin-like, if compared with the analogous 5-Et-$F_{1ax}$ [20] species. It must be realized that protons add to $F_{1ax}$ not at N-5 but at N-1 [27], which accounts for the much smaller oxidation power of $HsF_{1ax}$ as compared to $sF_{1ax}$.

A further component of the complex equilibria in the oxidized thiaflavin system is the solvated sulfonium cation $1HsF_{1ax}$-4a-X, X = OH, halogen or O-alkyl which corresponds by its chromophore to the 4a-protonated cation, whose spectrum is shown in Fig.1. This species is obtained pure in CF$_3$COOH solution (presumably X = OOCF$_3$) (cf. Fig.6). The bathochromic shift obtained upon N-1 protonation in the 4a-blocked system is characteristic, and it corresponds to the shift obtained upon C4a-protonation of the N-1-blocked system (cf. Fig.1).

Direct quantitative conversion of the reduced thiaflavin to the sulfoxide is, of course, achieved by peroxidation. Reduction of the sulfoxide under non-acidic conditions proved not to be feasible. This fact explains why thiaflavin functions as a reversible redox system at the radical and fully reduced levels only. The sulfoxide must be strongly bent according to this absorption spectrum and in agreement with the fact that in the planar state it would exhibit in the central subnucleus an antiaromatic 8 $\pi$ configuration.

Thiaflavodoxin of Peptostreptococcus elsdenii

When apoflavodoxin is added anaerobically to a solution of $sF_{5}FMNH$ in 0.1 M phosphate pH 7, no long-wavelength absorption typical of the radical is formed, even over prolonged periods. However, on mixing with air, protein-bound thiaflavin radical is produced slowly, as detected by its characteristic absorption spectrum (Fig.2). Thus it is clear that binding of the reduced $sF_{5}FMNH$ to the apoprotein results in a greater reactivity to O$_2$, since incubation of $sF_{5}FMNH$ without apoprotein results in no detectable radical formation or decay of the $sF_{5}FMNH$. This increase must be due to a flattening of the $sF_{5}red$H skeleton upon binding to the protein [19,28], which provides independent support for the flatness of the $HsFl_{red}$ conformation in natural flavodoxin [29].

Upon autoxidation the yield of protein-bound radical is not complete, being approximately 40% of that obtained by titration with a reactive nitroxide radical (see below). The lower yield of radical with O$_2$ appears to be due to overoxidation of the protein-bound radical by the product of O$_2$ reduction, O$_2^-$, since the yield is considerably increased (up to 70%) in the presence of superoxide dismutase. Catalase has no effect on the yield.

The optimum conditions for formation of protein-bound radical were found to be by oxidation with the strong Fe$^+$ acceptor, spirocyclohexyl porphyrinexide, a nitroxide radical [16]. Addition of nitroxide radical results in the immediate production of $sF_{1}$, both free and protein-bound. However, while the protein-bound radical is stable, the uncomplexed radical is not, and at pH 7.0, 15°C, has completely decayed after 40 min by irreversible dismutation. Thus, a mixture of apoflavodoxin and excess $sF_{5}FMNH$, in 0.1 M phosphate, pH 7.0, was titrated with nitroxide radical until no further production of enzyme-bound thiaflavin radical was obtained. This procedure yielded the spectrum shown in Fig.2, with $8_{565} = 4625$ M$^{-1}$ cm$^{-1}$.

Oxidation-Reduction Potential

The oxidation-reduction potential of the couple $sF_{1red}/sF_{1}$ is surprisingly high, as evidenced by the fact that equilibrium is established with the Fe(CN)$_6^{3-}$/Fe(CN)$_6^{4-}$ couple.

At pH values below 6.5, the radical decay is sufficiently slow so that direct spectrophotometric studies of this equilibrium may be made. For example, from a set of 12 individual experiments in 0.1 M phosphate pH 6.3, at 10°C, $8_{675}$ is 6000 M$^{-1}$ cm$^{-1}$ for $sF_{1}$ and the redox potential $E_m$, pH 6.3, is +0.45 V.

At pH above 7, radical disproportionation becomes sufficiently rapid so as to require determination of the radical by stopped-flow methods. In this way the oxidation rate of $sF_{1red}H$ by ferricyanide has been determined at pH 8.3 and 10°C as $k_{ox} = 1.83 \times 10^6$ M$^{-1}$.
s$^{-1}$ whereas for the radical and ferrocyanide we found
\[ k_{\text{red}} = 2.2 \times 10^5 \text{M}^{-1}\text{s}^{-1} \] which leads to \( K = k_{\text{red}}/k_{\text{ox}} = 0.12 \). Thus at pH 8.3, \( E'_{\text{m}} = +0.37 \text{V} \). From the pH dependence of this redox equilibrium, we calculate a pK \( \approx 7.0 \) for sFl$\text{red}$H and \( E'_{\text{m}} \), pH 7, of \( +0.38 \text{V} \).

In the thiariboflavin series, where instead of methyl the substituent in position 10 is ribityl, this pK drops to 6.2, probably due to the intramolecular hydrogen chelation between the ribityl side chain and N-1.

It is remarkable that the neutral thiaflavin radical exhibits the same type of extremely strong negative solvatochromism (cf. Fig. 2, Table 2) as does the natural blue flavosemiquinone HFl (\( \lambda_{\text{max}} \) in \( \text{H}_2\text{O} \approx 580 \text{nm} \), in benzene 655 nm) [30]. For the protein-bound flavosemiquinones \( \lambda_{\text{max}} \) is generally about 580 nm, which points to hydrophilic arrangement of the radical at the active site. This hydrophilicity is not due to an 'in-plane' hydration via N-5 -- H, as is shown by the present data on sFl, but must be a 'stacking interaction', in agreement with the data of Palmer and Mildvan [31] on proton relaxation rates of free and protein-bound flavin radicals.

The protein-bound thiaflavin radical, however, resembles the free radical in chloroform considerably more than in water.

Thus, both radicals, HFl and sFl, reflect a stacking contact with water by a hypsochromic shift of > 50 nm. When protein-bound, this contact is eliminated for sFl, but not for HFl. This interesting fact still awaits explanation.

It is disappointing that the redox potential of thiaflavin is so high, since this prevents, of course, enzymatic activity of thiaflavoproteins, even if the modified flavin is strongly bound by the enzyme, as in the present case. We intend, therefore, to construct thiaflavin derivatives of lower potential though not altered in steric shape.

This work was supported in part by grants from the Sonderforschungsbereich 138. We also appreciate the skilful technical assistance of Mr Michael Janda and valuable discussions with Drs S. Ghisla and G. Blankenhorn (Konstanz) and V. Favaudon (Paris).

REFERENCES

H. Fenner, R. Grauer, P. Hemmerich, H. Michel, and V. Massey

MATERIALS AND METHODS

Solvents and reagents were purchased from Merck, adding 2, 3, 5-trimethyl-pyridine and 2, 3, 5, 6-tetramethyl-pyridine.

Elemental analyses were performed on a Perkin Elmer 2400 spectrometer.

The 100% yield of the corresponding reduced thiourea was obtained by adding 30 % hydrogen peroxide solution, which was filtered off by suction. The precipitate was washed with diethylether and dried over phosphorus pentoxide (100°C, 13 Pa, 12 h).

**General observations:**

10% of the corresponding reduced thiourea was obtained by adding 30% hydrogen peroxide solution, which was filtered off by suction. The precipitate was washed with diethylether and dried over phosphorus pentoxide (100°C, 13 Pa, 12 h).

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