Reversible Transformation between the Oxidized and Reduced Forms of Redox Coenzyme Analogues

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Reversible transformation between 10-methylacridinium ion ($AcrH^+$) and 9,10-dihydro-10-methylacridine ($AcrH_2$) has been achieved by combining the photo-reduction of $AcrH^+$ by benzyl alcohol derivatives in MeCN at 298 K under irradiation of visible light of $\lambda > 360$ nm with the thermal oxidation of $AcrH_2$ by the corresponding benzaldehyde derivatives at 333 K. The photo-reduction of $AcrH^+$ by a benzyl alcohol derivative can also be combined with the photo-oxidation of $AcrH_2$ by dibenzyl disulphide under irradiation of light of λ 285 nm which corresponds to the absorption maximum of $AcrH_2$. Under continuous irradiation of light from a Xenon lamp, the $AcrH^+/AcrH_2$ redox pair acts as a photocatalyst for the oxidation of *p*-chlorobenzyl alcohol by dibenzyl disulphide to yield *p*-chlorobenzaldehyde and toluene-α-thiol. Reversible transformation between riboflavin-2′,3′,4′,5′-tetra-acetate (Fl) and the corresponding 1,5-dihydroflavin (FlH_2) has also been achieved by utilizing all possible combinations of thermal and photochemical reactions in controlling the direction of the redox reaction between Fl and benzenethiol derivatives, *i.e.*, the forward thermal reduction of Fl by benzenethiol derivatives combined with the reverse photo-oxidation of FlH_2 by the corresponding disulphides, the forward photo-reduction of Fl and the reverse photo-oxidation of FlH_2 under irradiation with light of different wavelengths, and the forward photo-reduction of Fl combined with the reverse thermal oxidation of FlH_2.

Nicotinamide adenine dinucleotide (NAD^+) is a major electron acceptor in the oxidation of various substrates.[1–3] In the oxidation of a substrate (SH_2), *e.g.*, alcohols, the nicotinamide ring of NAD^+ accepts two electrons and a proton, which are equivalent to a hydride ion, to yield the reduced form (NADH), equation (1).[1–3] This redox process is made reversible by the

$$NAD^+ + SH_2 \rightleftharpoons NADH + S + H^+ \quad (1)$$

presence of an appropriate enzyme, termed a dehydrogenase. The NAD^+/NADH coenzymes are released freely and consumed as redox co-substrates in the redox reaction [equation (1)]. In contrast, flavin coenzymes, which are also involved in a variety of dehydrogenation reactions, often stay tightly bound at the enzyme's active site.[4,5] There is not only a reductive half-reaction, *i.e.*, the reduction of the enzyme bound flavin (Enz-Fl) by a substrate (SH_2) [equation (2)], but also an oxidative half-

$$SH_2 + \text{Enz-Fl} \rightleftharpoons S + \text{Enz-}FlH_2 \quad (2)$$

reaction to regenerate Enz-Fl, *i.e.*, the oxidation of the reduced flavin (Enz-FlH_2) by an electron acceptor (X) [equation (3)].

$$\text{Enz-}FlH_2 + X \rightleftharpoons XH_2 + \text{Enz-Fl} \quad (3)$$

Thus, flavins act as catalysts for the dehydrogenation reactions of SH_2 by X. These enzymatic redox reactions [equations (1)–(3)] are reversible, and the transformation between the oxidized and reduced forms of redox enzymes occurs in both directions.[1–5] In the model systems of NAD^+/NADH and Fl/FlH_2, however, only one direction has been studied extensively; while considerable interest has so far been focused on either the oxidation of NADH model compounds by various substrates[6–8] or on the reduction of flavin analogues by substrates,[4,9–15] relatively little is known about the reduction of NAD^+ analogues by appropriate reductants except for dithionite[16–19] or the oxidation of the reduced form of flavins FlH_2 by oxidants except for dioxygen.[20] Moreover, there has been no report on the reversible transformation between the oxidized and reduced forms of the redox coenzyme analogues in the same redox reactions.

In this study, we report the reversible transformation between NAD^+ and NADH analogues in the redox reactions between benzyl alcohol derivatives and the corresponding aldehydes as well as the reversible transformation between Fl and FlH_2 analogues in the redox reactions between thiols and disulphides. Such reversible transformations have been made possible by utilizing appropriate photochemical systems for the forward and/or reverse direction in the redox reactions. A photocatalytic function of an NAD^+ analogue in the reduction of dibenzyl disulphide by *p*-chlorobenzyl alcohol is also reported.

Experimental

Materials.—10-Methylacridinium perchlorate ($AcrH^+$-ClO_4^-) was obtained by the addition of magnesium perchlorate to a methanol solution of 10-methylacridinium iodide, which was prepared according to the literature.[21] 9,10-Dihydro 10-methylacridine ($AcrH_2$) was prepared from 10-methylacridinium iodide by the reduction with $NaBH_4$ in methanol, and purified by recrystallization from ethanol as described elsewhere.[22] Riboflavin-2′,3′,4′,5′-tetra-acetate (Fl) was prepared by the reaction of riboflavin with acetic anhydride in pyridine, and purified by recrystallization from the ethanol and chloroform mixture.[23] The solubility of Fl in acetonitrile is much higher than that of riboflavin. Thiols (benzenethiol, *p*-methoxybenzenethiol, *o*-aminobenzenethiol, 2-naphthalenethiol, *p*-chlorobenzenethiol, 2,4,5-trichlorobenzenethiol, toluene-*p*-thiol, toluene-*m*-thiol, and toluene-α-thiol) and disulphides (diphenyl disulphide and dibenzyl disulphide) were obtained

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commercially. Acetonitrile which was also obtained commercially was purified and dried with calcium hydride by the standard procedure,[24] and stored under a nitrogen atmosphere.

Transformation between $AcrH^+$ *and* $AcrH_2$.—Typically, after an acetonitrile (MeCN) solution (2.0 cm^3) containing $AcrH^+ ClO_4^-$ (4.4×10^{-4} mol dm^{-3}) and *p*-chlorobenzyl alcohol (5.0×10^{-2} mol dm^{-3}) in a square quartz cuvette was deaerated thoroughly with a stream of argon and sealed, it was irradiated with visible light from a Ushio Model Ul-501C Xenon lamp through a Toshiba glass filter L-39 which transmits light of $\lambda > 360$ nm. The decrease in the $AcrH^+$ concentration was monitored by the visible spectrum (λ_{max} 358 nm, ε 1.8×10^4 dm^3 mol^{-1} cm^{-1}), using a Union SM-401 spectrophotometer. The cuvette was then immersed in a water bath which was thermostated at 333 K in the dark. The increase in the $AcrH^+$ concentration by the thermal reaction was also monitored by the visible spectrum of $AcrH^+$. This redox cycle, photoreduction of $AcrH^+$ and thermal oxidation of $AcrH_2$, was repeated several times. The products in the photo-reduction of $AcrH^+$ by *p*-chlorobenzyl alcohol were identified by the ^1H n.m.r. spectra, as follows. After an acetonitrile (CD_3CN) solution (0.60 cm^3) containing $AcrH^+ClO_4^-$ (6.0×10^{-2} mol dm^{-3}) and *p*-chlorobenzyl alcohol (6.0×10^{-2} mol dm^{-3}) in an n.m.r. tube was thoroughly degassed in vacuum by the successive freeze-pump-thaw cycles and sealed, it was irradiated for 7 h with the visible light of $\lambda > 360$ nm. The products, 9,10-dihydro-10-methylacridine and *p*-chlorobenzaldehyde, were analysed by comparing the ^1H n.m.r. spectra with those of authentic samples. The concentration of *p*-chlorobenzaldehyde (1.0×10^{-2} mol dm^{-3}) formed was the same as that of $AcrH_2$ (1.0×10^{-2} mol dm^{-3}). The ^1H n.m.r. measurements were carried out using a Japan Electron Optics JNM-PS-100 n.m.r. spectrometer (100 MHz).

The photo-oxidation of *p*-chlorobenzyl alcohol (5.0×10^{-2} mol dm^{-3}) by $AcrH^+ClO_4^-$ (1.0×10^{-4} mol dm^{-3}) in the presence of dibenzyl disulphide (1.0×10^{-3} mol dm^{-3}) in MeCN was carried out under irradiation of light of $\lambda > 360$ nm. The photoreduction of dibenzyl disulphide by $AcrH_2$ in the resulting solution was then performed under irradiation of monochromatized light from a Ushio Model UXL-157 Xenon lamp of a Hitachi 650-10S fluorescence spectrophotometer, λ 285 nm which corresponds to the absorption maximum of $AcrH_2$. This cycle, the photoreduction of $AcrH^+$ by *p*-chlorobenzyl alcohol and the photo-oxidation of $AcrH_2$ by dibenzyl disulphide, was repeated several times, when the decrease and increase in the $AcrH^+$ concentration were monitored by the visible spectrum. Continuous irradiation of a CD_3CN solution (0.60 cm^3) containing $AcrH^+ClO_4^-$ (4.3×10^{-2} mol dm^{-3}), *p*-chlorobenzyl alcohol (8.6×10^{-2} mol dm^{-3}), and dibenzyl disulphide (4.3×10^{-2} mol dm^{-3}) was carried out with light from a Ushio Model Ul-501C Xenon lamp without a filter for 24 h. The products, *p*-chlorobenzaldehyde (2.9×10^{-2} mol dm^{-3}) and toluene-α-thiol (5.8×10^{-2} mol dm^{-3}), were identified by the ^1H n.m.r. spectra, when no appreciable decrease in the $AcrH^+$ concentration was observed.

Transformation between Fl *and* FlH_2.—Typically, Fl (1.4×10^{-4} mol dm^{-3}) was added to a deaerated MeCN solution (2.0×10^{-3}) containing toluene-*m*-thiol (4.2×10^{-2} mol dm^{-3}) and tetrabutylammonium hydroxide (Bu_4NOH 3.3×10^{-4} mol dm^{-3}) in a square quartz cuvette, and the reaction at 298 K was monitored by the decrease in the absorbance at λ_{max} 442 nm due to Fl. The resulting reaction mixture was irradiated at 298 K with light from a Ushio Model Ul-501C Xenon lamp through a Toshiba glass filter UV-D33S which transmits light of 200 nm $< \lambda <$ 400 nm. The recovery of Fl in the photo-oxidation of FlH_2 by the disulphide formed in the thermal reduction of Fl by toluene-*m*-thiol was immediately recorded by the increase in the absorbance at λ_{max} 442 nm due to Fl. The products, FlH_2 and diphenyl disulphide, in the reduction of Fl (4.0×10^{-2} mol dm^{-3}) by benzenethiol (4.0×10^{-2} mol dm^{-3}) in the presence of Bu_4NOH (1.0×10^{-2} mol dm^{-3}) in CD_3CN (0.60 cm^3) were identified by comparing the ^1H n.m.r. spectra with the reported spectrum of FlH_2[25] and that of an authentic sample of diphenyl disulphide.

Results and Discussion

Reversible Redox Reactions of $AcrH^+/AcrH_2$ *with Benzyl Alcohol/Benzaldehyde Derivatives.*—We have previously reported that irradiation of the absorption band due to 10-methylacridinium ion ($AcrH^+$) in MeCN containing benzyl alcohol derivatives (X-$C_6H_4CH_2OH$) results in the reduction of $AcrH^+$ to yield 9,10-dihydro-10-methylacridine ($AcrH_2$), the corresponding aldehyde, and proton [equation (4)].[19] The

$$AcrH^+ + X\text{-}C_6H_4CH_2OH \xrightarrow[\lambda > 360\ nm]{h\nu} AcrH_2 + X\text{-}C_6H_4CHO + H^+ \quad (4)$$

singlet excited state of $AcrH^+$ has the strong oxidizing ability, judging from the largely positive reduction potential (2.3 V *vs.* SCE) in MeCN.[7,26] In addition, the excited state $^1AcrH^+$* has a relatively long lifetime (τ 31 ns).[26,27] In contrast, the NAD^+ coenzyme is non-fluorescent. However, NAD^+ is known to be strongly phosphorescent,[28] and the 1,N^6-etheno NAD^+ (ε-NAD^+), which is still enzymatically active, is fluorescent.[29] Thus, the $AcrH^+/AcrH_2$ redox pair may be considered as the $NAD^+/NADH$ analogues.

On the other hand, we have also reported that $AcrH_2$ can be oxidized by benzaldehyde derivatives in the presence of acid [equation (5)].[30] Since proton is produced in the photo-

$$AcrH_2 + X\text{-}C_6H_4CHO + H^+ \longrightarrow AcrH^+ + X\text{-}C_6H_4CH_2OH \quad (5)$$

reduction of $AcrH^+$ by benzyl alcohol derivatives [equation (4)], the back reaction, *i.e.*, the oxidation of $AcrH_2$ by benzaldehyde derivatives may occur thermally. In fact, the photoreduction of $AcrH^+$ by *p*-chlorobenzyl alcohol in MeCN under irradiation of visible light of $\lambda > 360$ nm is followed by the thermal oxidation of $AcrH_2$ by the photo-product, *p*-chlorobenzaldehyde at 333 K in the dark. This reversible transformation between $AcrH^+$ and $AcrH_2$ can be repeated as shown in Figure 1, where the decrease and increase in the $AcrH^+$ concentration in the redox cycle are plotted against the photochemical and thermal reaction time, respectively. Such reversible transformation between $AcrH^+$ and $AcrH_2$ has also been observed for the photo-oxidation of benzyl alcohol and *p*-methylbenzyl alcohol and the thermal reduction of the corresponding aldehydes.

Photo-oxidation of p-*Chlorobenzyl Alcohol by Dibenzyl Disulphide, Catalysed by the* $AcrH^+/AcrH_2$ *Redox Pair.*—The reducing power of $AcrH_2$ is weak as recognized by the slow rate of the thermal oxidation of $AcrH_2$ by *p*-benzaldehyde (Figure 1). However, the singlet excited state of $AcrH_2$ is known to be a much stronger reductant than the ground state.[7,21,31] Thus, the photo-oxidation of $AcrH_2$ by an appropriate substrate under irradiation of the absorption band due to $AcrH_2$ (λ_{max} 285 nm) may occur to regenerate $AcrH^+$. In such a case, $AcrH^+$ may act as a photocatalyst for the oxidation of *p*-chlorobenzyl alcohol by the substrate. In fact, irradiation of an MeCN solution containing $AcrH^+$ (1.0×10^{-4} mol dm^{-3}), *p*-chlorobenzyl alcohol

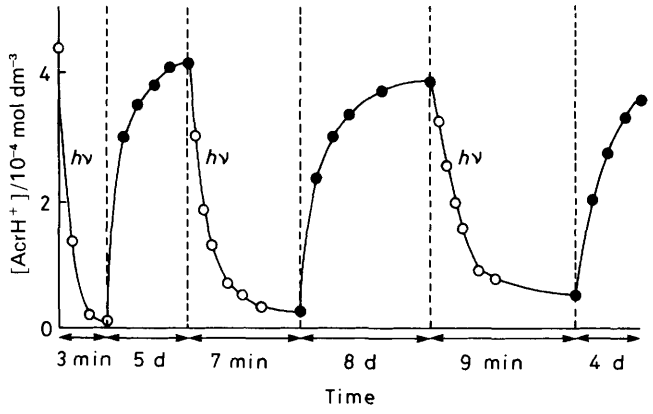


Figure 1. Repeated cycles for the decrease (○) and increase (●) in the $AcrH^+$ concentration in the photoreduction of $AcrH^+$ (4.4×10^{-4} mol dm^{-3}) by *p*-chlorobenzyl alcohol (5.0×10^{-2} mol dm^{-3}) in MeCN at 298 K under irradiation of visible light of $h\nu$ ($\lambda > 360$ nm) and the thermal oxidation of $AcrH_2$ by the corresponding aldehyde at 333 K, respectively.

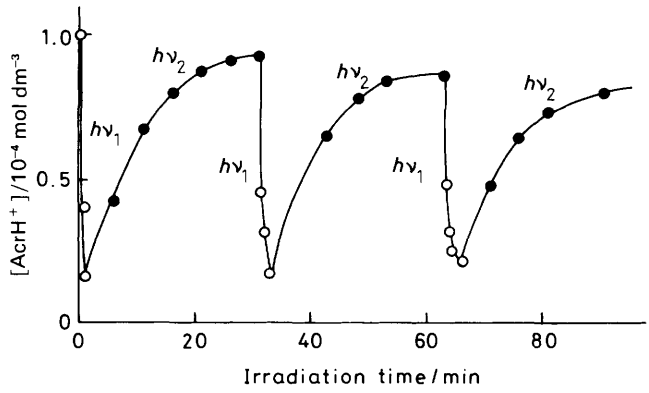
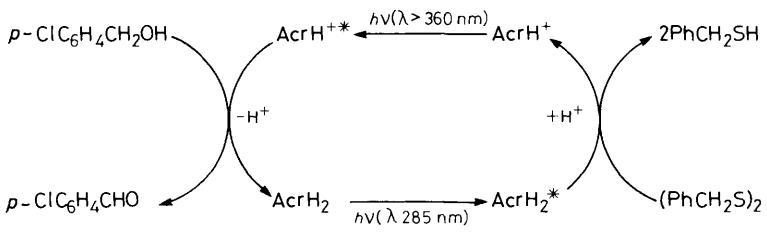


Figure 2. Repeated cycles for the decrease (○) and increase (●) in the $AcrH^+$ concentration in the photoreduction of $AcrH^+$ (1.0×10^{-4} mol dm^{-3}) by *p*-chlorobenzyl alcohol (5.0×10^{-2} mol dm^{-3}) in MeCN containing dibenzyl disulphide (1.0×10^{-3} mol dm^{-3}) and the photo-oxidation of $AcrH_2$ by dibenzyl disulphide at 298 K under irradiation of light of $h\nu_1$ ($\lambda > 360$ nm) and $h\nu_2$ (λ 285 nm), respectively.



Scheme.

(5.0×10^{-2} mol dm^{-3}), and dibenzyl disulphide (1.0×10^{-3} mol dm^{-3}) with visible light of $\lambda > 360$ nm results in the reduction of $AcrH^+$ by *p*-chlorobenzyl alcohol to yield $AcrH_2$ and *p*-chlorobenzaldehyde [equation (4)], and the subsequent irradiation of the reaction mixture with light of λ 285 nm which corresponds to the absorption maximum of $AcrH_2$ results in the regeneration of $AcrH^+$ by the photo-oxidation of $AcrH_2$ with dibenzyl disulphide to yield toluene-α-thiol [equation (6)]. This

$$AcrH_2 + (PhCH_2S)_2 + H^+ \xrightarrow[\lambda\ 285\ nm]{h\nu} AcrH^+ + 2\ PhCH_2SH \quad (6)$$

redox cycle can be repeated as shown in Figure 2. Thus, the appropriate choice of irradiation wavelengths make it possible to control the direction of the redox reactions, equation (4) or equation (6). Such reversible transformation between $AcrH^+$ and $AcrH_2$ has also been observed for the photo-oxidation of other benzyl alcohol derivatives (benzyl alcohol and *p*-methylbenzyl alcohol) by $AcrH^+$ and the photoreduction of aromatic disulphides (diphenyl disulphide and di-*m*-tolyl disulphide) by $AcrH_2$ under irradiation of light of $\lambda > 360$ nm and λ 285 nm, respectively.

When continuous irradiation of a CD_3CN solution (0.60 cm^3) of $AcrH^+$ (4.3×10^{-2} mol dm^{-3}), *p*-chlorobenzyl alcohol (8.6×10^{-2} mol dm^{-3}), and dibenzyl disulphide (4.3×10^{-2} mol dm^{-3}) was performed with light from a Xenon lamp without a filter for 24 h, *p*-chlorobenzaldehyde (2.9×10^{-2} mol dm^{-3}) and toluene-α-thiol (5.8×10^{-2} mol dm^{-3}) were formed with a 1:2 mol ratio, equation (7). In this case, the $AcrH^+/AcrH_2$ redox pair acts as a photo-catalyst for the oxidation of *p*-chlorobenzyl alcohol by dibenzyl disulphide as shown in the Scheme.

$$p\text{-}ClC_6H_4CH_2OH + (PhCH_2S)_2 \xrightarrow[AcrH^+/AcrH_2]{h\nu} p\text{-}ClC_6H_4CHO + 2\ PhCH_2SH \quad (7)$$

Reduction of Fl *by Benzenethiols.*—Thermal reduction of flavins by thiols have been studied extensively,[9–13] and it is well established that the reduction proceeds *via* general acid-catalysed thiolate attack on a flavin to form a C(4a)-adduct, followed by nucleophilic attack of a second thiolate on the C(4a)-adduct to yield the corresponding disulphide and 1,5-dihydroflavin.[9–13] Although ordinary flavin analogues are known to be reduced readily by aliphatic thiols, only electron-deficient flavins can be reduced by benzenethiol derivatives in aqueous solutions.[9,11] The reduction of ordinary flavins by benzenethiol has been made possible in a cationic micelle system[13] or in ethanol containing diazabicycloundecene.[12] Since the adduct formation becomes energetically more favourable with an increase in the basicity of benzene thiolate,[10] which is known to increase significantly in changing the solvent from H_2O to aprotic solvents such as Me_2SO,[32] the use of an aprotic polar solvent is expected to enhance the reactivity of benzene thiolate. In fact, the facile reduction of riboflavin-2′,3′,4′,5′-tetra-acetate (Fl) by benzenethiol derivatives occurs in the presence of Bu_4NOH in MeCN where the solubility of Fl is much higher than that of riboflavin. The formation of the corresponding 1,5-dihydroflavin (FlH_2) and disulphide were confirmed by the ^1H n.m.r. spectra (see the Experimental section), equation (8).

$$Fl + 2\ PhSH \xrightarrow[Bu_4NOH/MeCN]{} FlH_2 + (PhS)_2 \quad (8)$$

The rates of reduction of Fl by a large excess of thiols obeyed pseudo-first-order kinetics. The dependence of the observed pseudo-first-order rate constant k_{obs} at a fixed concentration of toluene-*m*-thiol (4.1×10^{-2} mol dm^{-3}) on the Bu_4NOH is shown in Figure 3. The k_{obs} value increases with an increase in the

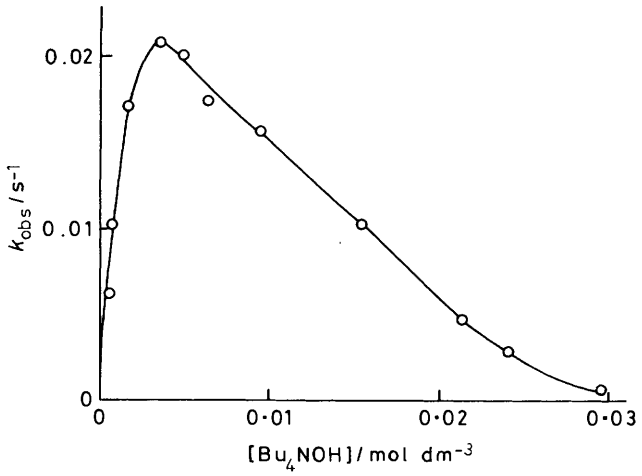


Figure 3. Plot of the observed pseudo-first-order rate constant (k_{obs}) *vs.* the Bu_4NOH concentration for the reduction of Fl (1.4×10^{-4} mol dm^{-3}) by toluene-*m*-thiol (4.1×10^{-2} mol dm^{-3}) in the presence of Bu_4NOH in MeCN at 298 K.

Table. Rate constants k_{obs} for the reduction of Fl (1.4×10^{-4} mol dm^{-3}) with aromatic thiols (4.0×10^{-2} mol dm^{-3}) in the presence of Bu_4NOH (3.3×10^{-3} mol dm^{-3}) in MeCN at 298 K.

Substrate	k_{obs}[a]/s^{-1}
p-Methoxybenzenethiol	1.6×10^{-1}
o-Aminobenzenethiol	3.6×10^{-2}
Naphthalene-2-thiol	2.6×10^{-2}
p-Chlorobenzenethiol	2.5×10^{-2}
Toluene-*p*-thiol	2.3×10^{-2}
Toluene-*m*-thiol	2.3×10^{-2}
Benzenethiol	9.7×10^{-3}
2,4,5-Trichlorobenzenethiol[b]	*c*

[a] The experimental errors are within ±10%. [b] The concentration was limited to less than 2.0×10^{-3} mol dm^{-3} because of the low solubility in MeCN. [c] No reaction.

Bu_4NOH concentration to reach a maximum at 4×10^{-3} mol dm^{-3} and then decreases in the higher concentrations. Such a maximal dependence is essentially the same as reported for the pH dependence of the reduction rates of flavins by thiols in aqueous solutions,[9-13] and thus consistent with the well established mechanism mentioned above. The k_{obs} values of various benzenethiol derivatives (4.0×10^{-2} mol dm^{-3}) are listed in the Table, where the k_{obs} value decreases with a decrease in the electron donor ability of the substituents when the basicity of the thiolate may also decrease.

Reversible Transformation between Fl *and* FlH_2.—Although photo-reduction of flavins by various substrates has been studied extensively,[14,15] very little is known about the excited states and the reactivities of the reduced flavins, 1,5-dihydroflavins. A reason for this could be the absence of a well-resolved structure in the near-ultraviolet absorption spectra. In addition, 1,5-dihydroflavins are known to be non-fluorescent in solution at room temperature, although they show marked fluorescence emission at 77 K in rigid media.[33] However, photochemical cleavage of the sulphur-sulphur bonds of aromatic disulphides has been well established,[34] and the thiyl radicals formed are known to be reduced readily by dihydroflavins to yield the corresponding thiols accompanied by formation of the oxidized flavins.[35] Thus, the photochemical reaction of FlH_2 with aromatic disulphides ArSSAr may occur to regenerate Fl and ArSH as shown in equations (9) and (10). In fact, irradiation of

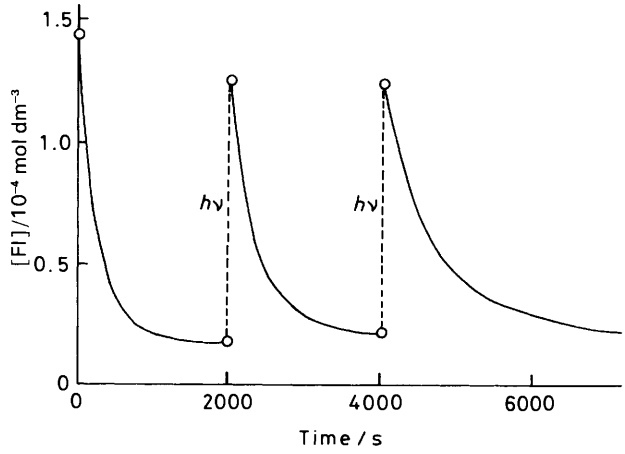


Figure 4. Repeated cycles for the decrease (——) and increase (– – –) in the Fl concentration in the thermal reduction of Fl (1.4×10^{-4} mol dm^{-3}) by toluene-*m*-thiol (4.2×10^{-2} mol dm^{-3}) in the presence of Bu_4NOH (3.3×10^{-4} mol dm^{-3}) in MeCN at 298 K and the photo-oxidation of FlH_2 by the corresponding disulphide under irradiation with light of 200 nm < λ < 400 nm, respectively.

$$ArSSAr \xrightarrow{h\nu} 2ArS^{\bullet} \text{ (light reaction)} \quad (9)$$

$$FlH_2 + 2ArS^{\bullet} \longrightarrow Fl + 2ArSH \text{ (dark reaction)} \quad (10)$$

the reaction mixture of the thermal reduction of Fl (1.4×10^{-4} mol dm^{-3}) by toluene-*m*-thiol (4.2×10^{-2} mol dm^{-3}) in the presence of Bu_4NOH (3.2×10^{-4} mol dm^{-3}) with light of 200 nm < λ < 400 nm which can excite the absorption band due to the disulphide (λ_{max} 243 nm) results in facile regeneration of the oxidized form (Fl). In this case, no semi-reduced flavin ($FlH^{\bullet}$ or $Fl^{-\bullet}$) has been observed during the reaction. Figure 4 shows the repeated cycles of the thermal reduction of Fl by toluene-*m*-thiol and the photo-oxidation of FlH_2 by the corresponding disulphide to regenerate Fl, equation (11).

$$Fl + 2(m\text{-}MeC_6H_4SH) \underset{h\nu(200 < \lambda < 400\ nm)}{\overset{\text{heat}}{\rightleftharpoons}} FlH_2 + (m\text{-}MeC_6H_4S)_2 \quad (11)$$

The reaction rate of thermal reduction of Fl by toluene-*m*-thiol can be slowed down by decreasing the thiol concentration. Thus, the thermal reduction of Fl (1.5×10^{-4} mol dm^{-3}) by *m*-toluenethiol (8.4×10^{-4} mol dm^{-3}) in the presence of Bu_4NOH (8.2×10^{-4} mol dm^{-3}) in MeCN occurred slowly but the rate was accelerated significantly under irradiation with visible light of λ > 360 nm which excites only the absorption band due to Fl (λ_{max} 442 nm). When the filter transmitting light of λ > 360 nm is replaced by that transmitting light of 200 nm < λ < 400 nm, the direction of the reaction is reversed and Fl is regenerated by the photo-oxidation of FlH_2 by di-*m*-tolyl disulphide. Thus, the direction of the redox reaction can be controlled by choosing the irradiation wavelength as shown in equation (12), and this cycle can be repeated as shown in Figure 5.

$$Fl + 2(m\text{-}MeC_6H_4SH) \underset{h\nu'(200 < \lambda < 400\ nm)}{\overset{h\nu(\lambda > 360\ nm)}{\rightleftharpoons}} FlH_2 + (m\text{-}MeC_6H_4S)_2 \quad (12)$$

When an MeCN solution containing Fl (1.4×10^{-4} mol dm^{-3}) and 2,4,5-trichlorobenzenethiol (1.0×10^{-3} mol dm^{-3}) which has no reactivity towards Fl thermally is irradiated with visible light of λ > 360 nm, the facile photoreduction of Fl by 2,4,5-trichlorobenzenethiol occurs as shown in Figure 6. In this case, however, the reverse reaction, *i.e.*, the oxidation of FlH_2 by the corresponding disulphide, occurs thermally at 298 K, and

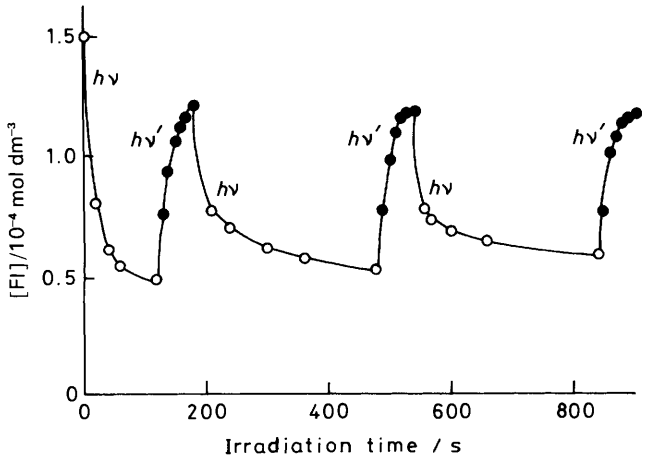


Figure 5. Repeated cycles for the decrease (○) and increase (●) in the Fl concentration in the photo-reduction of Fl (1.5×10^{-4} mol dm^{-3}) by toluene-*m*-thiol (8.4×10^{-4} mol dm^{-3}) in the presence of Bu_4NOH (8.2×10^{-4} mol dm^{-3}) in MeCN at 298 K and the photo-oxidation of FlH_2 by the corresponding disulphide under irradiation of light of *hν* ($\lambda > 360$ nm) and *hν′* (200 nm $< \lambda <$ 400 nm), respectively.

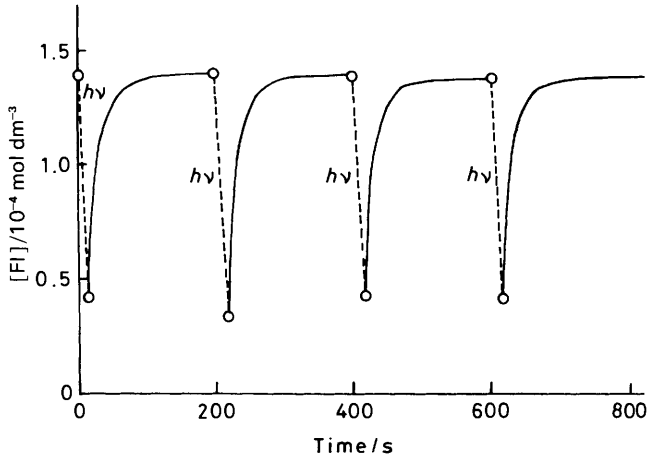


Figure 6. Repeated cycles for the decrease (– – –) and increase (——) in the Fl concentration in the photo-reduction of Fl (1.4×10^{-4} mol dm^{-3}) by 2,4,5-trichlorobenzenethiol (2.0×10^{-3} mol dm^{-3}) in MeCN at 298 K under irradiation with light of $\lambda > 360$ nm and the thermal oxidation of FlH_2 by the corresponding disulphide at 298 K, respectively.

the cycle of the photochemical reduction of Fl and the thermal oxidation of FlH_2 [equation (13)] can be repeated (Figure 6).

$$Fl + 2(2,4,5\text{-}Cl_3C_6H_2SH) \underset{298\ K}{\overset{h\nu(\lambda > 360\ nm)}{\rightleftharpoons}} FlH_2 + (2,4,5\text{-}Cl_3C_6H_2S)_2 \quad (13)$$

Such thermal oxidation of a dihydroflavin by diphenyl disulphide has been implicated in the lumiflavin-catalysed reduction of diphenyl disulphide by an NADH model compound, 1-benzyl-1,4-dihydronicotinamide in ethanol at 313 K.[36]

The two-electron reduction potential of riboflavin at pH 7 and 298 K in an aqueous solution has been reported to be −0.21 V (*vs.* NHE),[37] which is close to the two-electron oxidation potential of benzenethiol at pH 7 and 298 K in an aqueous solution, −0.19 V.[38] Thus, the free energy change of the redox reaction between Fl and benzenethiol may be close to zero. This may be the reason why all possible combinations of thermal and photochemical reactions in controlling the direction of the redox reaction between Fl and benzenethiol derivatives have been achieved by only choosing appropriate benzenethiol derivatives and irradiation wavelengths, *i.e.*, the forward thermal reduction of Fl by benzenethiols combined with the reverse photo-oxidation of FlH_2 by the corresponding disulphides [equation (11)], the forward photo-reduction and reverse photo-oxidation under irradiation of light of different wavelengths [equation (12)], and the photoreduction of Fl by 2,4,5-trichlorobenzenethiol combined with the thermal oxidation of FlH_2 by the disulphide [equation (13)].

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