# Coenzyme Models. Part 23.† Formation and Reactivity of the Stable ' Quinone Form ' of Flavin in Cationic Polymer Matrices‡

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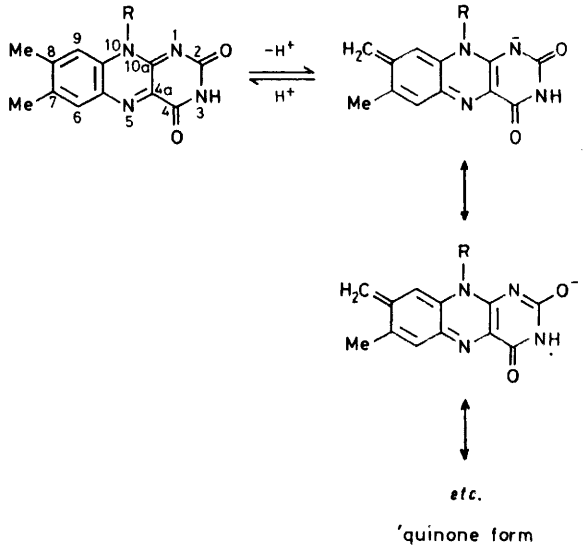
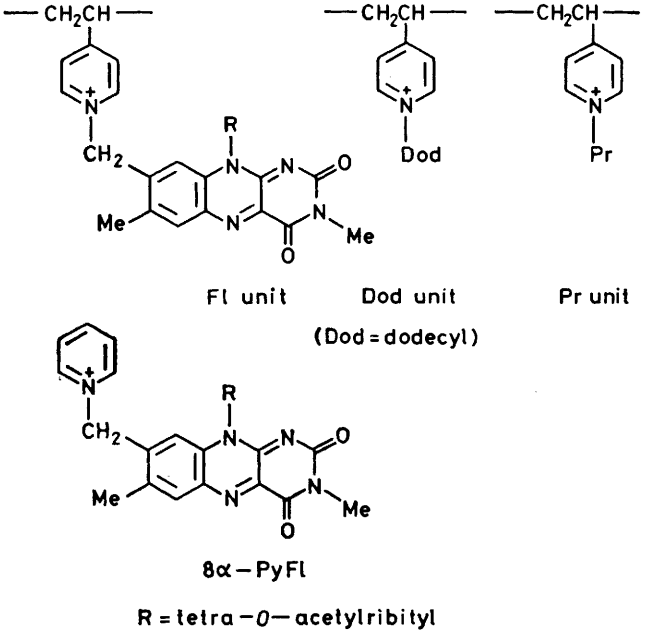
The flavin covalently linked to quaternised poly(4-vinylpyridine) *via* 8α-position afforded, in alkaline solution, a blue species absorbing at 622 nm. On the basis of spectral examination, the species is assigned to the ' quinone form ' of the flavin. The regioselective proton abstraction from the 8α-methylene is due to the enriched $OH^-$ concentration along the polymer chain, and probably also due to the stabilisation of the delocalised quinone form by the polyelectrolyte environment. The quinone flavin was insensitive to molecular oxygen and could oxidise neither NADH analogues nor thiols, indicating that the reactivity is markedly different from the normal flavin form.

CONSIDERABLE interest recently has centred around flavin-mediated oxidation–reduction reactions.[1] Model studies have established that the flavin usually employs the 4a-, 5-, or 10a-positions to execute the catalytic action.[1] Hemmerich *et al.*[2] suggested that the C-8 methyl group can be also classified as a functional group, since its acidity is as strong as that of the methyl group of *p*-nitrotoluene. The ' quinone form ' § of flavin has since been assumed to be an intermediate in H–D exchange,[4] Knoevenagel-type condensations,[5] and elimination from the 8α-position,[3] but there is no direct evidence for the existence of the ' quinone form ' of flavin except a dimeric analogue coupled at the 8α-position.[5]

We recently synthesised a flavin covalently linked to quaternised poly(4-vinylpyridine) *via* the 8-position, in order to estimate microenvironmental effects on the




reactivity of flavins.[6] Unexpectedly, we found that the polymer-bound flavin affords a blue species in alkaline solution. It was concluded on the basis of a spectral examination (see below) that the blue species is attributable to a ' quinone form ' of the flavin, and that the cationic polymer matrices stabilise it. To our knowledge, this is the first example of direct observation of the ' quinone form ' of a flavin.




## RESULTS AND DISCUSSION

The polymer-bound flavins (4VP-Fl-Dod-0 and 4VP-Fl-Dod-20: for the polymer composition see the Table) [6] and the monomeric flavin (8α-PyFl) [6] were treated in the absence of oxygen at 30 °C in two different aqueous solutions: solution A (pH 12.4, KOH) and solution B (pH 9.43, 0.02M borate buffer). In solution A 8α-PyFl showed

Composition of flavin-containing polymers (mol %) *

| Abbreviation | Fl unit | Dod unit | Pr unit |
|---|---|---|---|
| 4VP-Fl-Dod-0 | 6 | 0 | 88 |
| 4VP-Fl-Dod-20 | 6 | 20 | 67 |

* For the preparation and characterisation of these polymers see ref. 6.

† Part 22 is ref. 65. ‡ Abbreviations employed in this paper are: NADH, reduced form of nicotinamide adenine dinucleotide; 8α-PyFl, 3-methyl-8α-(1-pyridinio)tetra-*O*-acetylriboflavin bromide.

§ Edmondson and Singer [3] called the structure the ' quinhydrone form '.

spectral changes characteristic of the hydrolytic decomposition of the isoalloxazine ring, *i.e.* disappearance of the two absorption maxima (353 and 449 nm) and the appearance of a new maximum at *ca.* 400 nm.[7] The result establishes that nucleophilic attack of $OH^-$ on 8α-PyFl occurs at the usual electrophilic centre of the

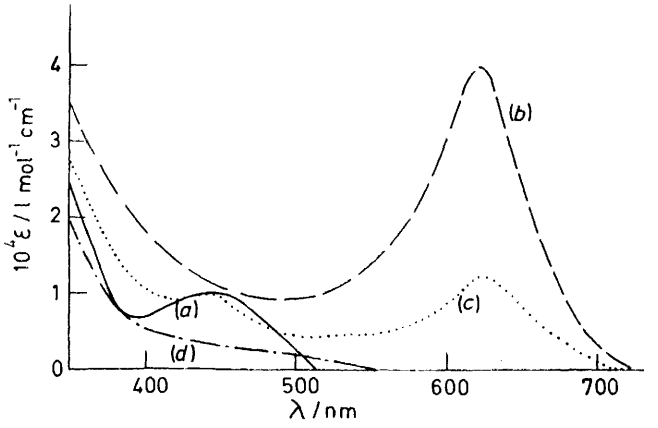



FIGURE Absorption spectra of 4VP-Fl-Dod-0: (*a*) oxidised flavin; (*b*) and (*c*), blue species obtained (*b*) solution A and (*c*) in solution B; (*d*) reduced flavin obtained by the reduction with 1-benzyl-3-carbamoyl-1,4-dihydroquinoline at pH 6.6 (0.02M phosphate buffer).

isoalloxazine ring, *i.e.* (4a) or (10a).[1,7] On the other hand, 4VP-Fl-Dod-0 ($\lambda_{max.}$ 448 nm, $\varepsilon_{max.}$ 100 40 l mol$^{-1}$ cm$^{-1}$) [6] in solution A gave rise to a new absorption maximum at 622 nm ($\varepsilon_{max.}$ 39 500 l mol$^{-1}$ cm$^{-1}$), with the absorption at 448 nm disappearing almost completely (Figure). In contrast to the well-known sensitivity of reduced flavin to oxygen, the spectrum of the blue species was hardly affected by the introduction of oxygen. The intensity at 622 nm decreased gradually with time, indicating the occurrence of further decomposition in the strongly alkaline solution A. The blue species disappeared instantaneously on the addition of an excess of acetic acid (three-fold excess over $OH^-$); the recovered intensity at 448 nm when the solution was made alkaline again was somewhat smaller than that of the original 4VP-Fl-Dod-0 solution.

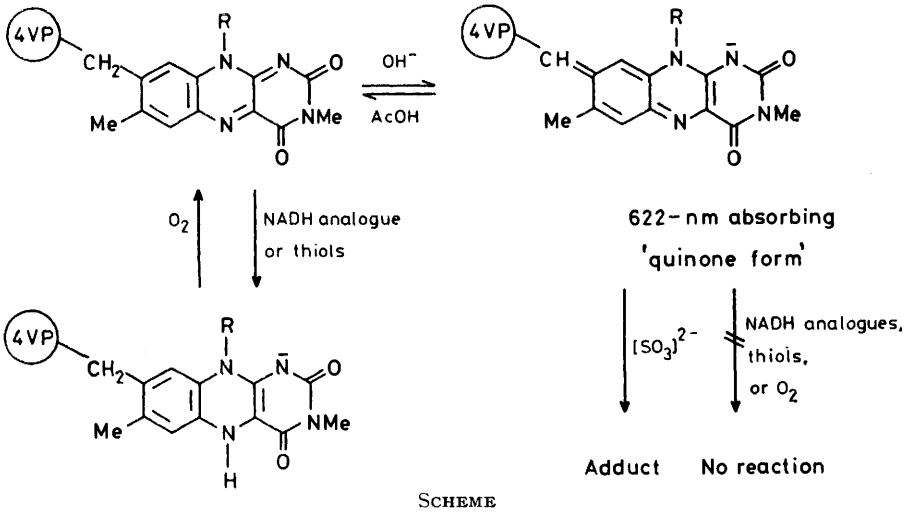
In solution B, the absorption maximum of 622 nm increased slowly with decreasing 448 nm absorption, and the intensities finally reached an equilibrium. The p$K_a$ value determined by equation (E1) was 9.80, where

$$pK_a = pH + \log[OD(pH\ 12.4) - OD(pH\ 9.43)]/OD(pH\ 9.43) \quad (E1)$$

OD = optical density at 622 nm

we assume that only the blue species exists in the reaction system at pH 12.4. The time-dependence of the optical-density values satisfied a first-order equation, and the rate constants determined by following the disappearance of the 448-nm peak ($1.68 \times 10^{-3}$ s$^{-1}$ for 4VP-Fl-Dod-0 and $2.65 \times 10^{-3}$ s$^{-1}$ for 4VP-Fl-Dod-20) were in good accord with those determined by following the appearance of the 622-nm peak ($1.72 \times 10^{-3}$ s$^{-1}$ and $2.62 \times 10^{-3}$ s$^{-1}$, respectively). Addition of an excess of acetic acid into the solution immediately regenerated the spectra of the starting polymer-bound flavins.

In the next stage, the reactivity of the blue species was evaluated. As reported previously,[6] the polymer-bound flavins rapidly oxidise NADH analogues, nitroalkane carbanions, and thiols. On the other hand, neither NADH analogues (1-benzyl-1,4-dihydronicotinamide and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline) [8] nor thiols (2-mercaptoethanol, thiophenol, and butane-1,4-dithiol) could be oxidised by the 622-nm absorbing species. It was found that only sulphite ion

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reacted with this species. The second-order rate constant for the blue species in 4VP-Fl-Dod-0 ($k_2$ = observed rate/[622-nm absorbing species] [$SO_3^{2-}$] = $1.83 \times 10^{-3}$ l mol$^{-1}$ s$^{-1}$) was 95 times slower than that for the 448-nm absorbing flavin (0.173 l mol$^{-1}$ s$^{-1}$). The result indicates that the blue species is less active as an oxidant.

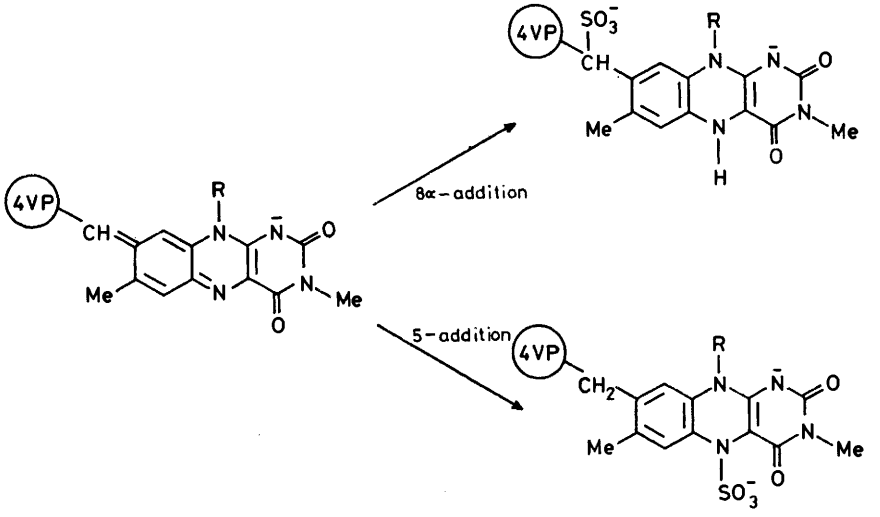
On the basis of the foregoing observations, the blue species is assigned to the polymer-supported analogue of the flavin shown in equation (E1).

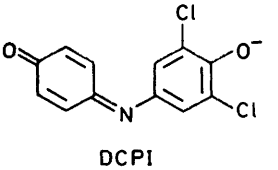
The ' quinone form ' is a result of the regioselective general base catalysis by $OH^-$ at the 8α-methylene; the cationic polymer environments then probably stabilises the electron-delocalised quinone structure.[9] The apparent acidity of the 8α-methylene group ($pK_a$ 9.80 for 4VP-Fl-Dod-0) is comparable with that of nitroalkanes (7.7—10.2).[10] As an oxidising agent, the ' quinone form ' is unable to oxidise NADH analogues and thiols, whereas as a reducing agent it does not react with oxygen, unlike ordinary reduced flavins, in spite of the electron-filled ring system. It should be noted here that all the reactivities described above are those of the quinone flavin bound to the cationic polymer matrices. We do not know whether the findings can be generalised to the reactivities of simpler quinone flavins in solution.

In general the position of attachments of flavin adducts can be conveniently determined by their characteristic u.v. spectra: thus, $\lambda_{max.}$ for adducts at the 5-position appears characteristically at 296—330 nm and that for adducts at the 4a-position at 360—370 nm.[11] In the present polymeric system, it was difficult to judge the position of the sulphite attack from the spectral data, since a strong absorption band from the poly(4-vinylpyridine) backbone overlapped the characteristic absorption area. On the basis of the resonance structure of the ' quinone form ', the 4a-position may be eliminated, leaving the 5- and 8α-positions. If the sulphite ion attacks the 8α-methylene, the resultant adduct would be the oxygen-sensitive 1,5-dihydroflavin, and the sulphite adduct was fairly stable to oxygen. The CPK model also suggests that the 8α-adduct is very sterically hindered. On the basis of these considerations, the sulphite adduct is probably attached to the 5-position.

It may be worth mentioning that the structure of the ' quinone form ' resembles 2,6-dichlorophenolindophenol (DCPI) which has an absorption maximum at 606 nm. Since NADH analogues are easily oxidised by DCPI,[12] the ' quinonide form ' with N and O atoms (*i.e.*, DCPI) must be more electron-deficient than that with N and C atoms (*i.e.*, quinone flavin).

Finally, it is interesting to consider why the reaction site of the polymer-bound flavin is the 8α-position and that of the monomeric 8α-PyFl is the 4a- or 10a-position. The result suggests that the cationic polymer matrix provides a regioselective field for the reaction of $OH^-$ with the covalently-linked reactant. It has been established that the local concentration of $OH^-$ along the cationic polymer chain is considerably enhanced in comparison to that in bulk water, and that some polymer catalytic effects are related with the enhanced $OH^-$ concentration.[13] If one accepts that the $OH^-$ concentration is larger in the vicinity of the cationic charge, it follows that the local $OH^-$ concentration sharply decreases the distance form the polymer chain increases. Although there is no useful literature to estimate the local $OH^-$ concentration as a function of distance from the polymer chain, the present example strongly suggests that the local $OH^-$ concentration around the 8α-methylene is very much higher than that around the 4a- or 10a-positions, the distance being only several Å. We believe that this finding would merit further investigation, since the regioselective behaviour involves a fascinating problem which could lead to the development of a new class of polymer catalysts.




DCPI

In conclusion, the quinone form of flavin, the existence of which has been supposed for a long time, has been observed in a polymer-bound form as a blue species. The importance of the polymer matrix in the formation and stabilisation of such intermediates is emphasised. We believe that further application of these ideas would

provide useful model systems for understanding the function of flavins and flavoproteins.

## EXPERIMENTAL

Preparations of flavin-containing polymers, 8α-PyFl, 1-benzyl-1,4-dihydronicotinamide, and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline, were as described previously.[6,8] 2-Mercaptoethanol, thiophenol, and butane-1,4-dithiol were distilled under nitrogen before use.

Spectral measurements were carried out in the absence of oxygen ($N_2$) at 30 ± 0.1 °C on a Hitachi 200 spectrophotometer. A modified Thunberg cuvette was employed to make measurements in the absence of oxygen; the detailed procedure of using the Thunberg cuvette has been described elsewhere.[14]

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