OXIDATION OF REDUCED FLAVINS BY QUINONES*

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Flavins are fairly widespread cofactors in enzymatic systems, but while many flavoproteins have been characterized and some studied mechanistically (1), there are many enzymatic reactions with flavins relative to which little is understood about the chemical system (2). Menadione and ubiquinones are known to be electron acceptors from flavoenzymes in some mitochondrial processes, and there are others currently under investigation (3). We wish to report here that quinones reoxidize dihydroflavins at extremely rapid rates, present data that rule out several possible pathways, and propose a likely mechanism for the reaction.

The addition of small aliquots of basic sodium dithionite to riboflavin in aqueous solution, or to lumiflavin and 3-methyllumiflavin in dimethylformamide, produces partially or fully reduced flavin. The absorbancies of these solutions at 440 or 450 nm follow a linear relationship with volume of dithionite solution added up to 1 mole equivalent, correcting for dilution

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where necessary. The peaks at 570 nm and 900 nm are observed, (these are respectively radical and the reduced-oxidized complex) but with the dilute solutions used here the absorbancies at 450 nm can be used to quantitatively determine the extent of flavin reduction to within 3%.

The addition of equimolar benzoquinone to 8 x 10^{-5} M dihydroriboflavin (Figure 1: R = ribityl, R' = H) in water in a Schlenck tube under argon results in the immediate reappearance of the oxidized flavin spectrum. The products of this reaction are cleanly riboflavin and hydroquinone.

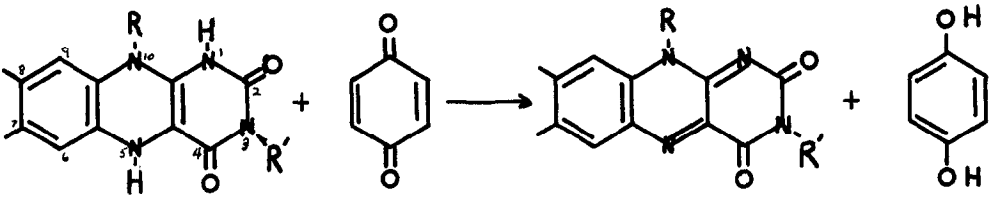


Figure 1

The reaction was not observably retarded by varying the pH from buffered pH 3 to pH 10 media, by substituting reduced lumiflavin (Figure 1: R = CH_3, R' = H) or 3-methyllumiflavin (Figure 1: R = R' = CH_3), nor by cooling the solutions to 0°. The reactions were run in dimethylsulfoxide and in dimethylformamide, again resulting in the essentially instantaneous reoxidation of the flavin. Less than one mole equivalent of quinone produced the corresponding equivalent amount of oxidized flavin.

The reaction between 1 x 10^{-5} M benzoquinone and 1 x 10^{-5} M dihydroriboflavin in aqueous solution was examined in an anaerobic Durrum Instruments stopped-flow spectrophotometer at 450 nm. At the fastest response time of the recording system which allowed observation after mixing (1 msec./div.), the mixing phenomenon (completed in approximately 2 msec.) was followed by

the fully oxidized flavin absorbancy. We were able to show that the deoxygenation was effective in that rapid mixing of aerated water with reduced flavin produced a curve for the reoxidation with a rate close to that previously reported (4), the O_2 reaction being much slower than the reaction with benzoquinone. Less than one equivalent of quinone immediately produced an exactly equal amount of oxidized flavin, then a very slow oxygen leak into the system.

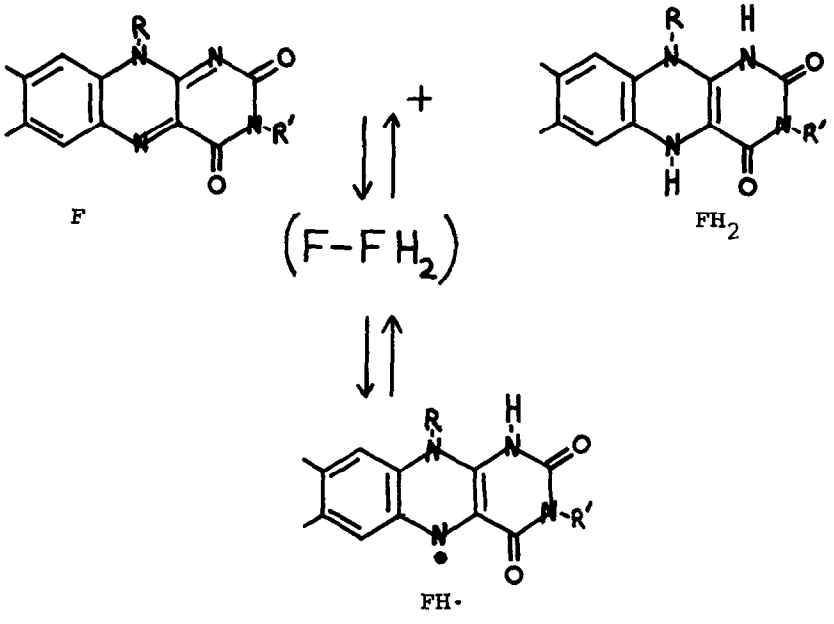
The overall reaction in these stopped-flow experiments was completed in less than 3 msec. Assuming a second order process, first order in each component, k_2 is greater than 3.3×10^7 $\underline{M}^{-1}$ $sec.^{-1}$. That is, k_2 is at least 1/30 of the diffusion controlled rate.

The difference in oxidation-reduction potential at pH 7 between the flavin couple (-0.21v) and benzoquinone (+0.29v) is 0.50 volt (5), making the reaction extremely favorable. In order to ascertain whether the reaction would be slowed down with weaker oxidizing agents we investigated the reaction with 1,4-naphthoquinone (+0.03v) and 9,10-anthraquinone (-0.27v) in Schlenck tubes in dimethylsulfoxide and dimethylformamide, at 5×10^{-5} $\underline{M}$ dihydroflavin and quinone. Naphthoquinone produced oxidized flavin within a second after mixing. In the case of anthraquinone, no oxidation of the flavin occurred in the course of one day. When air was admitted to this tube rapid oxidation by oxygen ensued.

Duroquinone (2,3,5,6-tetramethylbenzoquinone; E_o' at pH 7 is +0.05v, ref. 6) and 2,5-di-$\underline{t}$-butylbenzoquinone were also used in dimethylsulfoxide to reoxidize reduced 3-methyllumiflavin in the same solvent. No apparent retardation of the rate of flavin appearance was observed. In the course of their study

of the oxygen reoxidation of $FMNH_2$, Gibson and Hastings (4) noted that ferricyanide produced reoxidation to FMN faster than could be measured by stopped-flow. The estimated rate constant was greater than 10^8 $\underline{M}^{-1}$ $sec.^{-1}$.

In solution, it is known that the following equilibria take place, both lying towards the diamagnetic complex (7):



Our data exclude the formation of $F\text{-}FH_2$ from two FH· species since its known rate of formation (7) by this route is slower than the overall rate of the quinone reaction. If one electron oxidation occurs it must be followed by a further simple one electron removal from FH·. The stoichiometry of the reaction (cleanly 1:1) and the fact that one-half an equivalent of quinone immediately produces the one-half oxidized flavin spectrum makes such a stepwise mechanism kinetically indistinguishable from a two electron transfer.

Since; a) the reaction is extremely fast, b) quinones are

excellent π-electron acceptors, and c) dihydroflavins ought reasonably to be electron donors, an attractive possibility is electron transfer within an initially formed donor-acceptor complex. An association in which the dihydroflavin and quinone lie in a face to face manner appears attractive from the point of view of geometric considerations and hydrogen transfer.

Hydroquinones and oxidized flavins form rather stable 1:1 complexes in acidic solution (8). Under the various conditions of our experiments (acidity, solvent) 10^{-4} M flavin and 10^{-1} M hydroquinone showed no spectral changes indicative of complex formation.* However, the product resulting from the transfer of one electron or a hydride within a complex would most resemble the hydroquinone-protonated flavin system, and would have a real driving force for lowering the E_a to conceivably almost zero.

The flavoenzymes that catalyze the oxidation of NADH and NADPH by quinones are to be found in a great variety of cells. Is is known that flavins are efficient oxidizers of NADH (9) and dihydrolipoic acid (10),** and we now report the extreme rapidity and seeming non-selectivity of the oxidations of flavins by quinones. Even in the presence of oxygen, the availability of ubiquinone or menadione in a pool would favor the quinone pathway for reoxidation of free flavin, and conceivably that of the cofactor on many enzymes as well.

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* We have obtained some evidence of complex formation in a system in which N-5 of the dihydroisoalloxazine is alkylated so that oxidation is significantly retarded. This will be reported in a full manuscript.

** The reaction with mercaptans is general, M. J. Gibian and D. V. Winkelman, in preparation.

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