Tetrahedron Letters No.44, pp. 3901-3904, 1969. Pergamon Press. Printed in Great Britain.

THE OXIDATION OF MERCAPTANS BY FLAVINS

Morton J. Gibian and Dane V. Winkelman*

Department of Chemistry, University of California

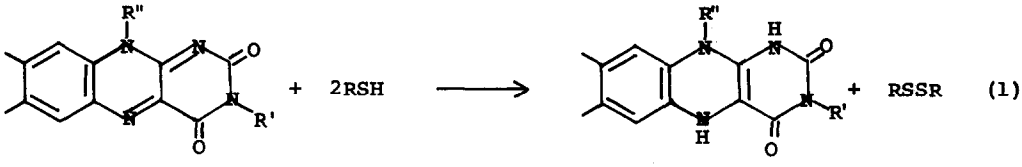
Riverside, California 92502

(Received in USA 14 July 1969; received in UK for publication 19 August 1969)

The reactions of flavins (isoalloxazines) with organic molecules are of interest as possible model systems for the catalytic reactions of vitamin B_2 (riboflavin) requiring proteins. The flavoenzyme lipoic acid oxidase catalyzes the oxidation of dihydrolipoic acid (6,8-dimercaptoctanoic acid) to lipoic acid, 3-(δ-valeryl)-1,2-dithiolane, and other flavoenzyme systems perform thiol to disulfide oxidations either as the primary reaction or as auxiliary processes to the main catalytic reactions(1,2). Gascoigne and Radda(3) reported that dihydrolipoic acid reacts with flavins in the absence of enzyme to produce lipoic acid and reduced flavin. It was noted that the reaction was first order in both flavin and in dihydrolipoic acid, and that it was also base catalyzed (the pH and buffer dependencies were obviously rather complicated). On the basis of this information and with the additional evidence that the relative rates for different flavins followed the same pattern as did the half-wave potentials for the two-electron polarographic reduction of the substituted flavins, the authors concluded that a two electron transfer occurs between the thiolate anion of dihydrolipoic acid and flavin to directly produce lipoic acid and reduced flavin. As part of our interest in flavin redox reactions(4) we wish to report our preliminary results of a survey of the reaction between flavins and mercaptans to produce dihydroflavins and disulfides according to eq 1.

---

*NSF-undergraduate research participant, Summer 1968.



We have found that this reaction is indeed quite general, and is efficient both intramolecularly (with 1,3-propanedithiol and 1,4-butanedithiol) and intermolecularly (with benzyl mercaptan and n-butyl mercaptan). The approximate rates and the products of these reactions are the same for riboflavin (R'=H, R"= ribityl), lumiflavin (R'=H, R"=CH_3), 3-methyllumiflavin (R'=R"=CH_3), and 3-carbethoxymethyllumiflavin (R'=$CH_2CO_2C_2H_5$, R"=CH_3).

Dihydroflavins (FH_2) are readily oxidized by molecular oxygen to flavins and hydrogen peroxide, which itself is known to be an efficient oxidizing agent for the oxidation of mercaptans to disulfides. Hence most of our reactions and all of our kinetics were carried out with the rigorous exclusion of air. By adding known quantities of oxygen to the reaction mixture, however, it has been possible to construct an essentially catalytic system, as described by equations 2-4.

$$F + 2RSH \longrightarrow FH_2 + RSSR \qquad (2)$$

$$FH_2 + O_2 \longrightarrow F + H_2O_2 \qquad (3)$$

$$H_2O_2 + 2RSH \longrightarrow 2H_2O + RSSR \qquad (4)$$

Followed by (2) ....., etc.

For the anaerobic reaction of excess benzyl mercaptan (R=$C_6H_5CH_2$-) and lumiflavin in aqueous methanol we obtained an 81% isolated yield of pure benzyl disulfide based on flavin (m.p. 68-69°, mixed m.p. 68-69.5°, identical by IR and NMR to authentic material). Control in the absence of flavin and air produces no reaction. In separate experiments known aliquots of air were added in successive portions following each flavin reduction by a large excess of mercaptan in a deaerated system under argon. In this case close to two disulfides

were obtained for each flavin reduced after summing over the several cycles.

With 1,4-butanedithiol in a dearated system we isolated a 17% yield of 1,2-dithiane after sublimation (this material readily polymerizes in solution, m.p. 31.5-32°, lit. 32-33°)(5). That normal dihydroflavin is indeed the final product of these reactions is demonstrated by: a) the spectrum of the final reaction mixture in both the benzyl disulfide and 1,4-dithiol systems, b) the rapid reoxidation in all systems by adding air, and c) thin layer chromatography of the dihydroflavin that has precipitated when high flavin concentrations were used in the benzyl mercaptan reaction.

Typical reaction conditions included a mixed solvent system consisting of 50% (by volume) water and dimethylformamide, dimethylsulfoxide, methanol, or acetonitrile. Carbonate buffer was used as the aqueous phase, which was then mixed with the organic solvent. The reaction was followed by observation of the decrease in oxidized flavin absorbance at 447 nm versus time. In all cases plots of log A_{447} versus time were linear except at high reactant concentrations where the disulfide precipitated after a short period of reaction. There was also a solubility problem with the mercaptans themselves in some cases. Under conditions where precipitation could be controlled, variation of the flavin concentrations over a 10-fold range while holding mercaptan constant showed excellent first order behavior in flavin. Variation in mercaptan at low (less than 5×10^{-3} M) concentrations showed first order dependence for both butanedithiol and benzyl mercaptan. The bleaching of flavin by the dithiol is only about two fold faster than the reaction with the same concentration of benzyl mercaptan, a fact which will be discussed below. All reactions were run in the dark except for kinetic runs in the very low intensity of the Cary 14 spectrophotometer. Taking points was equivalent to continuous monitoring on the Cary.

The reaction is base catalyzed. In acid and at neutral pH essentially no flavin bleaching and no mercaptan oxidation occurs. As one increases the basicity of the medium the reaction becomes more rapid, and then levels off at about pH 10. This can be interpreted as being due to the pK of the thiol group, and that the reaction entails prior ionization of the SH. Work is now in pro-

gress using soluble mercaptans and disulfides to accurately define the kinetics of these systems. An intriguing situation noted in this qualitative study was that the rate of reaction of dithiol and benzyl mercaptan was carbonate dependent when other variables were held constant. Our current efforts are directed toward defining whether this represents general base catalysis in this system.

Considering the generality of the reaction reported here, the similarity in rates between the intra- and intermolecular oxidations, and the basic catalysis observed, it is highly unlikely that one is first oxidizing one mercaptide ion to either a sulfenyl ion or a thiyl radical which finds another mercaptan or thiyl radical in aqueous organic solvent. It would seem much more likely from several of the observations given here that first mercaptide attacks the flavin to form an intermediate in which the sulfur is much more susceptible to nucleophilic attack than in a thiol. Fast attack upon this intermediate by RS^- (or RSH) to produce RSSR and FH^- would explain our data. It is required that; a) attack by the first mercaptide ion produce an intermediate with the spectrum of reduced flavin or b) attack by the second mercaptan be rapid.

Acknowledgments: Financial support of this work by the Public Health Service (GM-15100) and by the Intramural Research Fund of the University of California is gratefully acknowledged.

References:

(1) Flavins and Flavoproteins, BBA Library, Vol. 8, E. C. Slater, Ed., Elsevier Publishing Company, New York, N.Y. 1966.

(2) Biological Chemistry, H. R. Mahler and E. H. Cordes, Harper and Row, New York, N.Y., 1966.

(3) I. M. Gascoigne and G. K. Radda, Biochim. Biophys. Acta, 131, 498 (1967).

(4) M. J. Gibian and J. A. Rynd, Biochem. Biophys. Res. Commun., 34, 594 (1969).

(5) J. A. Barltrop, P. M. Hayes, and M. Calvin, J. Am. Chem. Soc., 76, 4348 (1954).