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The Paramagnetic Resonance Absorption of a Semiquinone of FMN

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Recently Beinert¹ has presented strong evidence for the existence of a semiquinoid compound appearing as an intermediate in the oxidation-reduction reactions of flavinmononucleotide (FMN). In collaboration with Gutowsky and Rutledge he succeeded in obtaining a paramagnetic resonance signal characteristic of a free radical from FMN reduced by Zn in an acid 50% aqueous ethanol solution¹. They failed to obtain any signal with a pure aqueous solution*, because of the high dielectric losses in liquid water. It is, however, of the greatest importance to be able to investigate the paramagnetic resonance of this and similar substances as the postulated semiquinones of FAD, DPN and TPN when they are dissolved in water, the milieu of the enzymatic reactions in which they are supposed to take part^{2,3}.

By freezing the samples, which diminishes the dielectric losses greatly, and

* Personal communication.

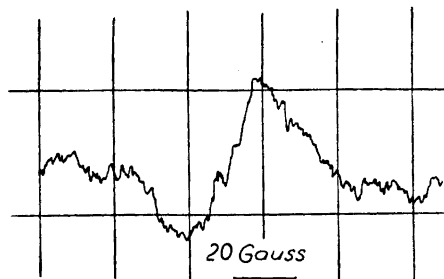


Fig. 1. Derivative of paramagnetic resonance absorption of 160 μ M FMN in 1 N HCl after reduction by solid Zn for 8 min and subsequent rapid cooling to about 80° K.

working at liquid N₂ temperatures, which improved the sensitivity, it has now been possible to record intense resonance signals from the semiquinone of FMN in dilute solutions. The FMN was dissolved in 1 N HCl and reduced by metallic Zn, until the color was brownish. A 0.20 ml sample was transferred to a small quartz tube, which immediately was dipped into liquid N₂. The solution rapidly solidified and was cooled down to 80° K. The sample was then transferred into the precooled high-Q cavity of the spectrometer⁴ operated at 9 400 Mc and the spectrum was recorded (Fig. 1). It did not change over a period of 30 min at liquid N₂ temperature. No signal could be obtained from FMN before adding Zn or after reoxidation in air, nor from a sample without FMN but reacted with Zn. The g-value of the resonance is practically identical with that of the free radical diphenylpicryl hydrazyl. No further signals could be seen in the region 2 600 to 3 900 gauss and no hyperfine structure has thus far been resolved.

Comparison of the surfaces under the absorption curve obtained by integration of Fig. 1 and that of a known amount of diphenylpicryl hydrazyl reveals that the concentration of free radicals is roughly half the initial concentration of FMN. This would appear to dispel any doubt that a semiquinone intermediate of FMN is really formed under the conditions described.

The technique used here will be of great value in cases when the intermediate is shortlived, since the low temperature will practically lock the reaction at a desired

stage. Work is in progress to correlate the amount of FMN-semiquinone to the absorption curves of the reaction mixture in the visible region under various conditions and to investigate whether the semiquinone can be formed from FMN, when it is bound to the apoprotein of the old yellow enzyme.

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