

Effect of Polysorbate 80 and of Sodium Lauryl Sulfate on the Photodegradation of Flavin Mononucleotide: A Kinetic Study with Electron Spin Resonance

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Abstract □ A kinetic study on the effect of polysorbate 80 and sodium lauryl sulfate (SLS), on the photolytic degradation of flavin mononucleotide (FMN), was carried out through measurements of the electron spin resonance signal of the semiquinone, one of its intermediate degradation products. A plateau concentration of the semiquinone was first achieved with the aid of a constant light source. The decay was subsequently measured in the dark and was found to obey first-order rate of reaction. A comparative study of the rate of formation of semiquinone in the presence of polysorbate 80 and of SLS was then calculated from the plateau level and the decay rate constants in each condition. Both SLS and polysorbate 80 were found to accelerate the formation of the semiquinone derivative, then increase the rate of photodegradation of FMN.

Keyphrases □ Flavin mononucleotide—photodegradation □ Surfactant effect—flavin mononucleotide photodegradation □ Electron spin resonance—photodegradation determination

Michaelis *et al.* (1) have demonstrated that the reduction of riboflavin and riboflavin-5-phosphate (flavin mononucleotide, FMN), to their leucoforms, pass through a semiquinone intermediate. The presence of this semireduced stage was first demonstrated by Khun and Wagner-Jauregg (2), who found a red intermediate in the reduction of riboflavin with zinc and hydrochloric acid. Michaelis *et al.* (3) employing potentiometric titrations have also found evidence for the existence of semiquinone in neutral and a weak alkaline solution of riboflavin.

The absorption characteristics, and the ESR signal of the semiquinone have been studied by Beinert (4) and by Gibson (5). An absorption band at 570 m μ was observed and attributed to the semiquinone stage. An ESR signal of 17.6 gauss was recorded and identified as coming from the semiquinone. Commoner and Lippincott (6), reported the ESR signal of semiquinone to be 20.8 gauss. Oster and Bellin (7) claimed that the photoreduction of riboflavin to semiquinone

must pass through an intermediate stage, *i.e.*, triplet stage.

The effect of macromolecules on photodegradation of riboflavin was studied by Kostenbauder *et al.* (8). They have demonstrated that nonionic and anionic macromolecules enhance the photodegradation of riboflavin.

In order to further elucidate the mechanism of this phenomenon, a study of the kinetics of photodegradation of FMN in the presence of macromolecules, with the aid of ESR was undertaken.

MATERIALS AND METHODS

FMN (flavin mononucleotide : riboflavin-5-sodium phosphate),¹ polysorbate 80,² and sodium lauryl sulfate³ (SLS) were used.

Equipment—The light source was a 500-w. lamp-tungsten lamp with filters.⁴ Maximum transmittance of the filter combination was at 460 m μ . The effective band width was from 425 to 490 m μ . An ESR spectrometer was used.⁵

A 10⁻² M solution of FMN in phosphate buffer of 0.05 M and having a pH of 6.8 was prepared. The solution was then purged with nitrogen for 10 min. Next, the solution was transferred into an ESR cell of 0.03 mm. diameter and stoppered. The filters were inserted in the front of the cavity and the light source adjusted at a distance of 40 cm. from the cavity. The cavity temperature was held by the thermoregulator at 25°.

Prior to illumination, the instrument was set at a modulation amplitude of 100 gauss/min. which did not broaden the observable hyperfine splitting, and the microwave power level was set at 2.5 db, so that it did not give any detectable saturation.

The recorder was then switched on for 5 min. Subsequently the light source was turned on and the experiment continued until the semiquinone signal, which appeared at 3,360 gauss, achieved a constant plateau. The light source was then turned off but the tracing was continued until the signal became undetectable at field intensity of 3,360 gauss.

The same experiment was repeated with a solution of FMN in a similar buffer, but containing 1% and subsequently 4% polysorbate 80 w/v. The experiment was also repeated with an FMN solution containing 0.001 M and 0.04 M sodium lauryl sulfate (SLS).

RESULTS AND DISCUSSION

The size and shape of the ESR signal is shown in Fig. 1. Its size of 20.8 gauss, and the intensity of the magnetic field in which it generated, as well as its fine structures, correspond to the semiquinone signal described previously (4-6). The intensity of the ESR signal is directly related to the concentration of the semiquinone in the solution. Plotting the height of the signal against time gives, therefore, relevant information of the kinetics of its formation and decay.

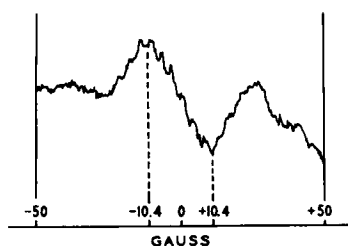


Figure 1—Electron spin resonance spectra of FMN in phosphate buffer.

¹ Merck & Co., Rahway, N. J.

² Tween 80, Atlas Chemical Co., Wilmington, Del.

³ City Chemical Corp., New York, N. Y.

⁴ Corning No. 3-73 and No. 5-57.

⁵ Varian model 4250B.

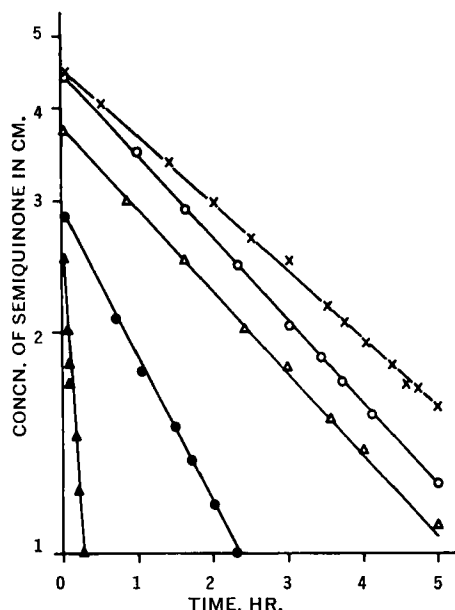


Figure 2—Effect of varying concentrations of polysorbate 80 and SLS on the decay of the electron spin resonance signal of semiquinone. Key: x, FMN 10^{-2} M; O, FMN 10^{-2} M + polysorbate 80, 1%; ●, FMN 10^{-2} M + polysorbate 80, 4%; Δ, FMN 10^{-2} M + SLS 0.001 M; ▲, FMN 10^{-2} M + SLS 0.04 M.

The effect of polysorbate 80 and SLS on the decay of the semiquinone radical is demonstrated in Fig. 2. Both polysorbate 80 and SLS increase the rate of decay of the semiquinone radical. In every experiment the decay proceeded as first-order reaction. The velocity constant of the decay reaction (K) increased from 0.206 hr.^{-1} without macromolecules to $K = 0.45 \text{ hr.}^{-1}$ in the presence of 4% polysorbate 80, and to $K = 3.8 \text{ hr.}^{-1}$ in the presence of 0.04 M SLS.

It may be concluded, therefore, that the decay of the semiquinone radical is enhanced in the presence of polysorbate 80 as well as SLS. When the formation of the semiquinone radical was plotted against time, a constant plateau was always reached—indicating equilibrium between the rate of formation of new semiquinone radical and its rate of decay. It was also noted that in the presence of 4% polysorbate 80 and SLS 0.04 M, equilibrium was reached at a lower concentration of semiquinone radicals. Since irradiation conditions were constant in all experiments, and decay rate was found to be a concentration-dependent first-order reaction, the rate of formation of the semiquinone radical could be calculated from its decay rate (K), at the concentration of the achieved plateau (C) i.e., $C \times K =$ rate of formation.

Since the height of the signal is directly related to the concentration of the semiquinone, but the conversion factor is unknown, the results are expressed in millimeters per hour.

The rate of formation of the semiquinone radical was found to be 9.27 mm./hr. without macromolecules. It was increased to 12.85 mm./hr. in the presence of 4% polysorbate 80 and to 95.0 mm./hr. in the presence of 0.04 M SLS (Table I).

The effect of polysorbate 80 and SLS on the rate of formation of the semiquinone radical was investigated by an indirect method by Kostenbauder *et al.* (7). An acceleration of photodegradation of

Table I—Polysorbate 80 and Sodium Lauryl Sulfate Effect on Semiquinone Formation Rate

Solution Studied	K Constant of Degradation Rate, hr.^{-1}	Concn. Semiquinone Radical at Equilibrium, mm.	Rate of Formation of the Semiquinone Radical
	K	C	$C \times K$
FMN 10^{-2} M	0.206	45.0	9.27
FMN 10^{-2} M, polysorbate 80, 1%	0.25	45.0	11.25
FMN 10^{-2} M, polysorbate 80, 4%	0.45	28.5	12.82
FMN 10^{-2} M, SLS 0.001 M	0.27	37.0	9.99
FMN 10^{-2} M, SLS 0.04 M	3.8	25.0	95.00

riboflavin that was related to an increased formation of semiquinone radical was found. Through the use of ESR, a more exact quantitation of the semiquinone radical was possible, as well as a direct study of the kinetics of formation and the degradation.

It is concluded that the enhanced photodegradation of riboflavin in the presence of polysorbate 80 and SLS is due to a combined effect of an increased formation of the semiquinone, and an enhanced rate of its degradation. The exact mechanisms involved need further study. It may be suggested that the trapping of the short-lived triplet intermediate by macromolecules is involved in the increased rate of formation of semiquinone radical in the presence of polysorbate 80 and SLS. Contact between the macromolecules and the semiquinone radical seems also to catalyze its subsequent reduction to lumichrome.

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