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EFFECT OF CERTAIN STABILIZERS ON PHOTOBLEACHING OF RIBOFLAVIN SOLUTIONS*

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ABSTRACT

The influence of certain stabilizers on the aerobic photobleaching of buffered riboflavin phosphate solutions exposed to fluorescent light was investigated. Disodium ethylenediamine (EDTA) demonstrated the greatest stabilizing effect followed by thiourea, methylparaben, DL-methionine and sodium thiosulfate. The photostabilizing effect of these agents increased as their concentrations increased. Other stabilizers enhanced the photostability of riboflavin solutions with lesser and varying degrees. The pH and buffer species of the solutions appeared to influence the rate of photodegradation of riboflavin solutions in the presence or absence of EDTA.

INTRODUCTION

The photosensitivity of riboflavin was first reported in 1932 by Warburg and Christian (1). Since then several reports have been published on its photochemistry. It has been suggested

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that the photodecomposition of riboflavin proceeds from the lowest triplet state of the flavin and involves intramolecular hydrogen-transfer from the ribityl side chain with the subsequent formation of lumichrome and/or lumiflavin depending on the basicity of the solutions (2-6). In deoxygenated aqueous solution, riboflavin has been reported to be reduced to dihydroriboflavin or leucoflavin on exposure to light (7).

Stabilization of riboflavin solutions to light is pharmaceutically and medicinally important. However, it appears from literature reports that very little work has been done on the photostabilization of riboflavin solutions. Therefore, it appeared worthwhile to study the effect of certain stabilizers on the photolysis of riboflavin in buffered solutions.

EXPERIMENTAL

Materials: Riboflavin-5' phosphate sodium was obtained from Sigma Chemical Company. Other chemicals were obtained from commercial sources in pharmaceutical or analytical grade and were used without further purification.

Procedure: Volumes of 2 mg% riboflavin sodium phosphate in the various buffers with and without the photostabilizers were exposed to fluorescent light as previously described (8). Samples were withdrawn at designated time intervals and assayed spectrophotometrically at 445 nm (Hitachi UV-Visible Spectrophotometer, Model 100-60) against appropriate blanks. No interference with the assay was demonstrated by any of the stabilizing agents under investigation. The analysis was done on duplicate samples and the difference between duplicates was usually 0-1.5%.

DISCUSSION OF RESULTS

Effect of Stabilizers: It appears from Figure 1 and Table 1 that EDTA produced the greatest stabilizing effect among all the stabilizers tested. Increase of the photostability of riboflavin by more than 70% was demonstrated by EDTA, thiourea, methylparaben, DL-methionine and sodium thiosulfate in a descending order. EDTA increased the

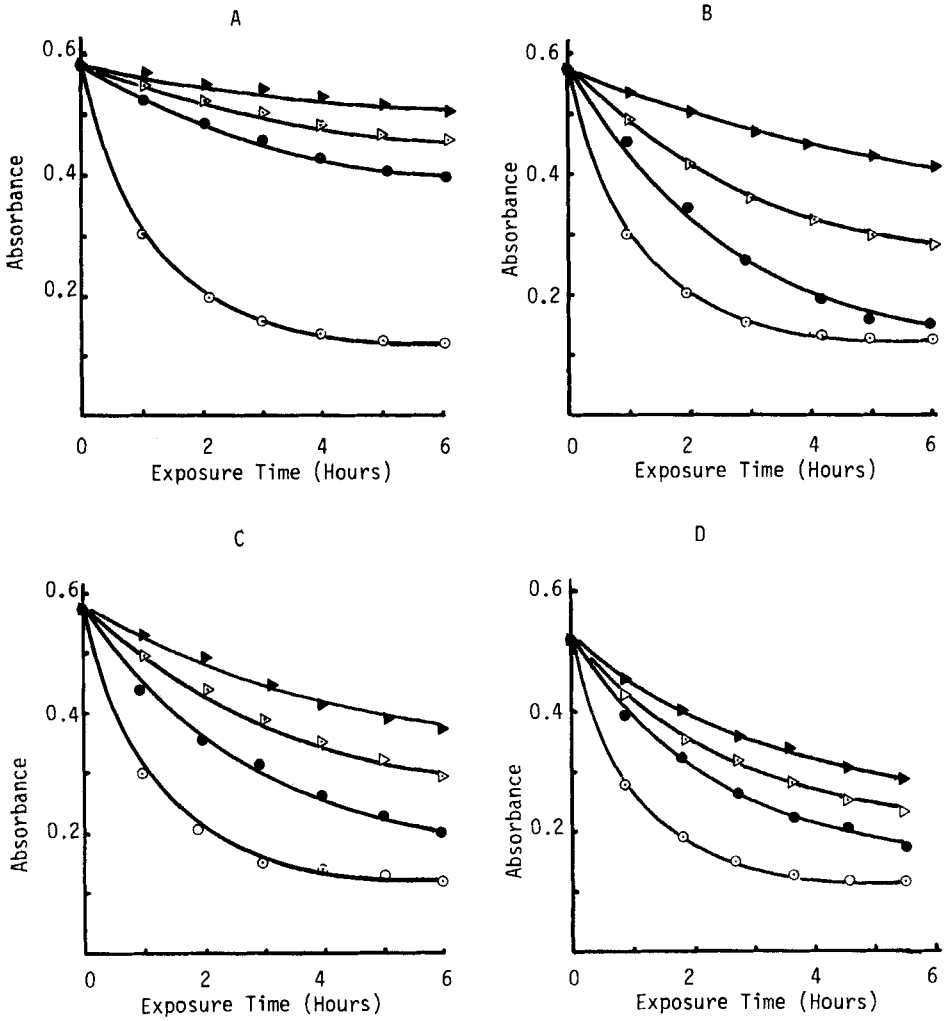

FIGURE 1

Photodegradation of Riboflavin Solution in Phosphate Buffer of pH 7 in the Presence of Various Concentrations of the Stabilizers. A - EDTA; B - Thiourea; C - Methylparaben; D - DL-Methionine;⊙ Without Stabilizer; ●0.05%; ▽ 0.15%; ▼ 0.3%.

TABLE 1

Effect of Various Stabilizers on the Photobleaching of Riboflavin Solution in Phosphate Buffer of pH 7

Stabilizer (0.3%)	Rate Constant Kx 10^2 hr^{-1}	% Increase in Stability*
None (Control)	55.8	—
EDTA	2.1	96.2
Thiourea	6.6	88.2
Methylparaben	7.6	86.4
DL-Methionine	13.2	76.3
Sodium Thiosulfate	15.1	72.9
Ribonucleic Acid	22.7	59.3
Reduced Glutathione	41.2	26.2

* Calculated from $\frac{Kc-Ks}{Kc} \times 100$, where Kc and Ks are the reaction rate constants for the control solution without stabilizers and the sample solution with the stabilizers respectively.

stability of riboflavin in phosphate buffer of pH 7 by 96.2% whereas sodium thiosulfate insreased its stability by 72.9%. Increasing the concentrations of these stabilizers resulted in an increase of their photostabilizing effect.

It is interesting to note that after photolysis, riboflavin solutions stabilized with EDTA or DL-methionine showed two distinct layers. The upper layer was yellow and the bottom layer was colorless. These solutions were shaken to allow for the homogenous distribution of color before its measurement at 445 nm. This could be attributed to the formation of dihydroriboflavin or leucoflavin which was subsequently oxidized to riboflavin by atmospheric air as postulated by Nickerson and Strauss (9).

TABLE 2

Effect of pH on the Photostability of Riboflavin in Phosphate Buffers with and without 0.15% EDTA.

pH	% of Riboflavin Remaining after Exposure to Light					
	1 hr.		2 hrs.		6 hrs.	
	a	b	a	b	a	b
9	44	88	35	83	23	70
8	49	94	37	90	24	77
7	57	95	39	91	25	79
4.5	61	91	40	85	25	70

a: without EDTA b: with EDTA

The photostabilizing effect of methyl paraben for riboflavin solutions can be attributed to its phenolic group which was reported by Shin and his associates (10) to play a significant role in the photostabilizing effect of some phenols for riboflavin through lowering its quantum efficiency under aerobic conditions.

Yest nucleic acid in a concentration of 0.5% was found by Kostenbauder et al. (11) to considerably inhibit the fading of riboflavin in phosphate buffer of pH 6.8 when exposed to light from a tungsten lamp for two hours. The stabilizing effect was attributed to the dark reaction between the ground state dye molecule and the adenine moities of nucleic acid. In our study, yeast ribonucleic acid was found to increase the stability of riboflavin solution in phosphate buffer of pH 7.0 by 59.3% when exposed to fluorescent light for two hours.

Effect of pH: Table 2 shows that photodegradation of riboflavin solution in the presence or absence of EDTA is pH dependent. Solutions appeared to be most stable at pH 7-8 when phosphate buffers were used. Riboflavin solution has been reported to rapidly de-

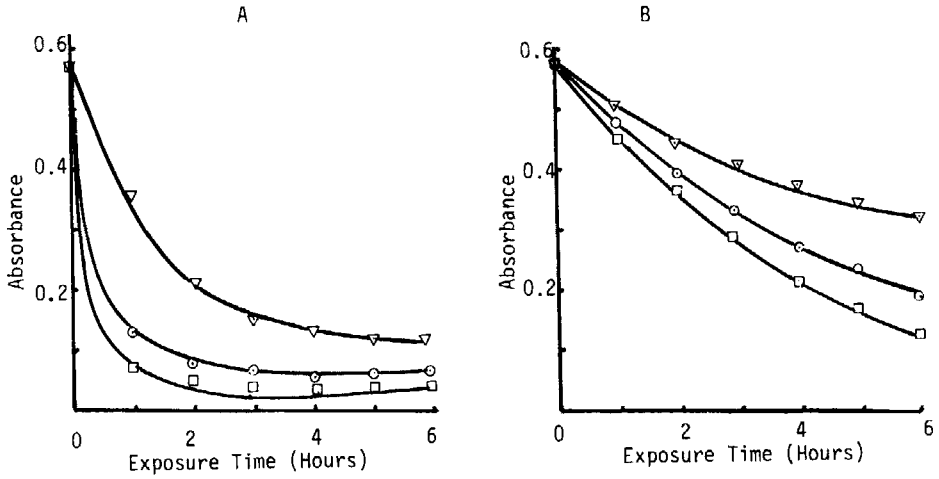

FIGURE 2

Photodegradation of Riboflavin Solution in Various Buffers of pH 4.5. A - Without EDTA; B - With EDTA; □ Citrate; ○ Acetate; ▽ Phosphate.

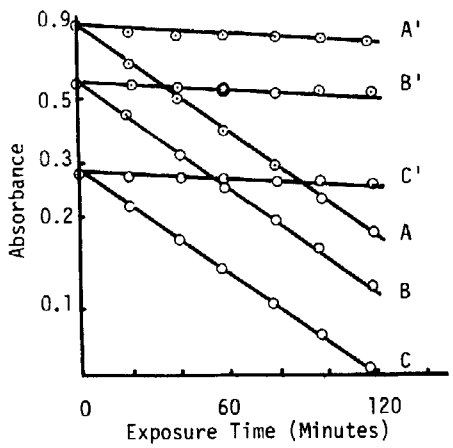

FIGURE 3

Effect of Concentration of Riboflavin on its Rate of Photodegradation in Phosphate Buffer of pH 7. A, B and C: Solutions without EDTA; A', B' and C' : Corresponding Solutions with 0.15% EDTA.

grade by light especially in the presence of alkali (12). Moreover, photolysis of riboflavin solutions has been found to be subject to general acid-base catalysis (4).

Effect of Buffer Species: It appears from Figure 2 that riboflavin solution in the presence or absence of EDTA was most stable in phosphate buffer of pH 4.5 and least stable in citrate buffer of the same pH. The catalytic effect of citrate ions on the photofading of FD&C Blue No. 2 which formed a semiquinone intermediate as riboflavin was reported by Asker and Collier (13).

Order of Reaction: It appears from Figure 1 that riboflavin solution containing no stabilizers degraded according to first-order kinetics. This was confirmed by using three concentrations of riboflavin as shown in Figure 3. However, there was deviation from the first-order kinetics when the reaction approached completion after 5 hours. This result is in agreement with the finding of Kostenbauder and his associates (11). Such result may be expected if a photoproduct or an intermediate quenches excited molecules (14) or if a product of the reaction or an intermediate absorbs incident light as in the case of riboflavin (11). In the presence of stabilizers, on the other hand, the reaction rates appeared to follow zero-order kinetics as can be seen in Figure 1 especially during the initial periods of exposure to light. Therefore, the initial rate constant calculated in this study was based on the two-hour exposure period. It was determined from the most linear portions of the zero-order plot of concentration versus time. This degradation rate constant was used to calculate the percentage increase in stability of riboflavin produced by various stabilizers.

CONCLUSIONS

The effect of certain stabilizers on the photobleaching of riboflavin solutions in various buffers was determined. EDTA demonstrated the greatest photostabilizing effect. pH and buffer species appeared to influence the rate of photodegradation of riboflavin in the presence or absence of EDTA.

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