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Lumiflavin-sensitized Photooxygenation Accelerated by Adenine. II. The Photolysis of ^{14}C-labeled Reactants

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Synopsis. Radioactive tryptophan, histidine, methionine, and guanine were photolyzed in the presence of lumiflavin either with or without adenine, and the reaction products were compared chromatographically. Adenine accelerated these lumiflavin-sensitized photooxygenation reactions, and no effect of adenine on the reaction products was shown.

In a previous paper,[1] it was reported that the lumiflavin-sensitized photooxygenation of indoles is greatly accelerated by the addition of adenine when the reactants are present in very low concentrations. At that time, fluorometry was succesfully used as a highly sensitive method to follow the reaction; however, there remained an open question of whether or not adenine simply accelerates the oxygenation of indoles without altering the basic pattern of the reaction. In the present work, the effects of adenine on the lumiflavin-sensitized photooxygenations of tryptophan, histidine, methionine, and guanine, and on the reaction products have been examined by the use of ^{14}C-labeled reactants.

Experimental

The lumiflavin was prepared as has previously been described.[1] *dl*-Tryptophan-3-^{14}C (3.76 mCi/mmol), *l*-histidine-^{14}C (U) (216 mCi/mmol), and *l*-methionine-1-^{14}C (5.81 mCi/mmol) were purchased from the Daiichi Pure Chemicals Co., While guanine-8-^{14}C hydrochloride (5.4 mCi/mmol) was obtained from the New England Nuclear Co.

The photolysis was carried out as has previously been described (at 435.8 nm and 30 °C).[1] After the paper chromatography of the reaction mixtures on Toyo Roshi No. 51A paper, the autoradiograms were prepared by exposing Sakura Industrial X-ray film (type N) (8.4×30.5 cm) for several weeks. The radiochemical counting was carried out using a liquid-scintillation counter (Beckman LS-250) for sections of the paper-chromatograms in 5-ml solutions of 2,5-diphenyloxazole in toluene (4 g/l), or conventionally using a gas-flow counter (Aloka TRM-1) with a paper-chromatogram scanner for each strip of the chromatograms. The initial concentration of tryptophan-3-^{14}*C* was determined by fluorometry,[1] while the other ^{14}C-labeled reactants were measured by the use of the liquid-scintillation counter.

Results and Discussion

The reaction mixtures of the lumiflavin-sensitized photooxygenation of 10 μM tryptophan-3-^{14}*C* in the absence and in the presence of adenine were developed on filter paper with 1-butanol–acetic acid–water (4:1:2). The autoradiograms for the samples at different reaction periods (from 20% to more than 90% decomposition) showed that tryptophan decomposed to several reaction products and that the patterns of the products were not changed with the degree of the decomposition. Therefore, Fig. 1 shows the autoradiograms for the reaction mixtures in which the photolysis was almost completed in the presence and in the absence of adenine. In both chromatograms, six spots of the reaction products were observed; no difference was detected between the patterns of these reaction products with and without adenine. Similar results were obtained when 1-butanol saturated with water was used as the developing solvent. Thus, it was concluded that adenine does not alter the final products in spite of its great accelerating effect on the reaction rate. At pH 8.0, where the reaction quantum yield was about twice as much as that at pH 7.2, the photoproducts also consisted of plural species which were not altered whether adenine was present or not.

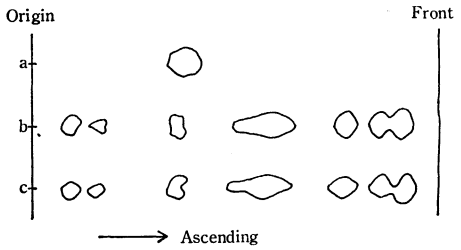

Fig. 1. Autoradiograms for the photoproducts of 10 μM tryptophan-3-^{14}*C* sensitized by 2.5 μM lumiflavin in the absence and presence of adenine.
a) Adenine 0. Before irradiation.
b) Adenine 0. Irradiation 280 min (92% decomp.).
c) Adenine 0.2 mM. Irradiation 82 min (98% decomp.).

In the previous work,[1] the reactions were followed by fluorometry, by which indoles were analyzed with high accuracy even in micromolar concentrations without any interference from adenine. The radioactive-iso-

Table 1. Accelerating effect of adenine on the lumiflavin-sensitized photooxygenation of several ^{14}C-labeled reactants

Reactant	Reaction time (min)	Fraction of reactant remaining	
		without adenine	with adenine[a]
Tryptophan	27	0.81	0.25
(10 μM)	82	0.53	0.02
Histidine	75	—	0.56
(0.8 μM)	165	0.88	0.24
Methionine	21	—	0.6
(1.0 μM)	64	0.9	0.2
Guanine[b]	90	0.72	0.16
(8.0 μM)	150	0.56	0.05

Lumiflavin 2.5 μM, phosphate buffer 0.05M, at pH 7.2.
a) Adenine 0.2 mM. b) At pH 6.9.

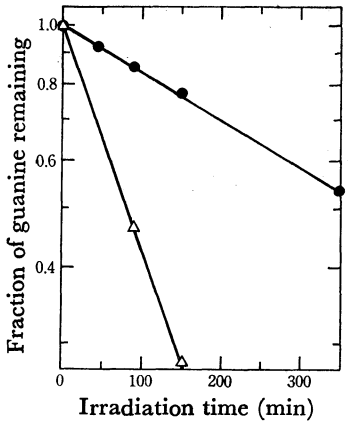

Fig. 2. First order plots for the photooxygenation of 8.0 μM guanine-8-^{14}C sensitized by 2.5 μM lumiflavin in 0.05 M phosphate buffer at pH 6.9.
Adenine: ● 0 mM and △ 0.2 mM.

tope-tracer method is good for the kinetical treatment of the reaction as well as for the product analysis. Therefore, the effect of adenine on the photolysis of nonfluorescent ^{14}C-labeled reactants under dilute conditions was examined. Among the nonfluorescent amino acids which undergo photodynamic action, histidine and methionine were chosen; the effect of the addition of adenine on the lumiflavin-sensitized photooxygenation of these reactants was investigated. The results are summarized in Table 1. The accelerating effect of adenine was observed as in the case of tryptophan.

Besides several amino acids, guanine also undergoes photodynamic action.[2] It was similarly examined whether or not the photodecomposition of this purine derivative is affected by another purine derivative, adenine. Since guanine is hard to dissolve in water (5 mg/l), guanine-8-^{14}C hydrochloride was first dissolved in 0.1 M hydrochloric acid and then diluted. The pH value of the sample solution containing 8.0 μM guanine and 2.5 μM lumiflavin was adjusted to 6.9 with a 0.05 M phosphate buffer. The accelerating effect of the addition of 0.2 mM adenine on the photosensitized decomposition of guanine was also observed on the autoradiograms, as is shown in Fig. 2 and Table 1. When guanine was decomposed by equal quantities of both solutions with and without adenine, the effect of adenine on the reaction products was also examined. Successive reactions were observed to occur, but the identity of the products was observed at every stage of the reaction.

On the basis of the results mentioned above, it was concluded that the addition of adenine accelerates the lumiflavin-sensitized photooxygenation of not only tryptophan, but also of many reactants, and that it does not alter the reaction type. Similarly, the enhancement of photodynamic action may take place *in vivo*, because flavin dyes and adenine are general substances in bioorganisms.

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References

1) A. Yoshimura and S. Kato, This Bulletin, **46**, 1141 (1973).

2) A. D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids," Pergamon Press, Oxford (1964), p. 162.