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Methylene Blue에 의한 Guanine의 광분해 현상

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Methylene Blue-Catalyzed Photodecomposition of Guanine

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ABSTRACT

1) The photodecomposition rate of guanine being catalized by methylene blue was 53.7% in contrast with 9.3% that of dark control for 180 min.

2) In guanine control, the decomposition rate was very low. For 180 min., the rate was 8.1% in illuminated sample and 3.9% in non-illuminated sample.

3) The decomposition rate of methylene blue was obviously interfered by the existence of guanine. In guanine and methylene blue mixture solution, the net decomposition rate, excluding that of dark control was 39.2% and in dye only solution, it was 48.5%.

INTRODUCTION

The photochemistry of nucleic acids is of special interest because of their important biological functions; their very high extinction in the ultraviolet region of the spectrum which is most efficient in the production of mutagenic, lethal, and other biological functions. It should be emphasized that the photochemistry of nucleic acids is of interest not only from a biological, but also from a physicochemical point of view.

A good deal of work has therefore been done in which the primary aim has been to achieve appreciable degradation and to elucidate the nature of the chemical changes in a system resulting from its exposure to light(Shugar, 1960).

And a knowledge of the photochemical behavior of nucleic acid derivatives is an obvious prerequisite to any attempts to interpret the effects of radiation on polynucleotide chains. It is well known that guanine residue in deoxyribonucleic acid(DNA) or ribonucleic acid(RNA) can be photodecomposed in the presence of methylene blue, thiopyronine and proflavine(Ponce-De León and Cabrera-Juárez, 1970; Singer and Fraenkel-Conrat, 1966; Von Vunakis *et al.*, 1962). And there were reported about dye catalyzed photodecomposition of guanosine (Sastry and Gordon, 1966), photodynamic effect of the carcinogen, 3,4-benzpyrene on *Paramecium caudatum*(Epstein *et al.*, 1963), and Benzo(α)pyrene on *Escherichia coli* (Harrison and Raabe, 1967) and physicochemical properties of complex between DNA and Antibiotics such as actinomycin D (Kersten *et al.*, 1966). The mechanism how these sensitizers such as acridine dye, benzopyrene, and some antibiotics such as actinomycin D react with guanine residue of

DNA or RNA has not been clearly known.

Ponce-De León and Cabrera-Juárez reported that the absorption spectra of the illuminated DNA with dye showed apparently small changes in comparison with the untreated ones. On the other hand, Von Vunakis could not follow the photodynamic reaction by spectral changes in nucleic acid because the hyperchromicity caused by the photodynamic denaturation of the acids masked the hypochromicity caused by destruction of guanine in DNA.

The object of this paper is to confirm whether guanine base can be photodecomposed by methylene blue.

To obtain the decomposition rate of guanine, absorption spectra were measured.

MATERIALS AND METHODS

For preliminary experiment, two kinds of guanine and dye mixture solution were prepared as follows. One solution was made in 0.1 N HCl solution and the other in 0.1 N NaOH solution. After illumination for 3, 6, 9, 12, and 24 hours, reaction mixtures were paper-chromatographed to separate guanine, dye, and the other product from the mixture. A 50 μl of reaction mixture was applied to Whatman No. 1 paper (15×57cm) and developed with isopropanol: 2 N HCl (V/V). After dried, each spot was detected under the UV lamp, separated, and extracted with 3.5 ml of 0.1 N HCl and 0.1 N NaOH, respectively.

Optical density of supernatant fraction was measured at 250 nm. The guanine content of each sample which had been extracted from the spot of irradiated acidic reaction mixture didn't show any detectable variations. On the other hand, there were much decreases of guanine content in alkaline reaction mixture. To determine the decreasing rate of guanine content in alkaline solution, this experiment was performed.

Reaction mixture solutions:

Three series of mixture were perpared as follows;

1. The first group of reaction mixtures; Pipet precisely 2.5ml of guanine (Wako pure chemical, Japan) solution containing 240 μg/ml in 0.1 N NaOH solution into a 1×13 cm screw-cap test tube. And add 0.5ml of methylene blue (Katayama chemical, Japan) (2×10^{-4}M) to this tube. The final concentration of guanine is 200 μg/ml and of methylene blue is 3.3×10^{-5}M.

2. The second group of reaction mixtures; Pipet 2.5ml of guanine solution and 0.5ml of distilled water instead of methylene blue solution into cap-test tube. This process was carried out in order to calibrate the decreasing rate of guanine without methylene blue but only by light illumination.

3. The third group of reaction mixtures; Only 2.5ml of 0.1 N NaOH solution without guanine and 0.5 ml of methylene blue solution are mixed in the cap-test tube. This reference process was performed because blue color of methylene blue was decolorized rapidly without guanine by illumination in our preliminary test.

Illumination:

The illumination was provided by a 200 W tungsten lamp (Sylvania) from under the sample with 6,000 foot-candles. Through the experiment the test tubes were laid in the glass chamber containing cool water (15°C) in cold room. The test tubes checked with time interval of 30 minutes from 0 to 180 minutes. The dark control tube was wrapped in aluminum foil. The types of mixture solution and checked time are shown in Table 1.

Table 1. Each test tube removed one by one with time interval, diluted in dark room, if necessary, and its optical density was measured.

Time (Min.)	Guanine with dye		Guanine only		Dye only	
	Illuminated	Dark	Illuminated	Dark	Illuminated	Dark
0	+	+	+	+	+	+
30	+	+			+	
60	+	+	+	+	+	+
90	+	+			+	
120	+	+	+	+	+	+
150	+	+			+	
180	+	+	+	+	+	+

Measuring of Optical density:

Absorption spectra were measured by Shimadzu spectrophotometer and Hitachi spectrophotometer at ultraviolet range(200 to 300 nm) and visible range(600 to 700 nm). To measure the optical density of guanine in UV range, 200μl of each sample was taken and diluted 1 in 25 with 4.8 ml of 0.1 N NaOH and to measure that of methylene blue undiluted sample was used directly. But optical densities of the first group in UV range was included both absorption spectra of guanine and methylene blue. So to obtain net photodecomposition rate of guanine, optical density of the third group of samples(methylene blue only solution) in UV range was measured in undiluted state because of low absorption spectra of methylene blue in UV range. And then it was excluded from the absorption spectra of the first group.

The concentration of guanine and methylene blue could be calculated by means of molar extinction coefficient.

$$E=\frac{A}{C\times 1}$$

E; Molar extinction coefficient

A; Optical density in maximum peak

C; Concentration in mole per liter

1; The thickness of cell(1 cm)

E_{max} of guanine in *p*H 13 was 9.3×10^3 and that of methylene blue was 4.7×10^4 (λ_{max} of guanine in *p*H 13 was 274 nm and that of methylene blue was 668nm).

RESULTS AND DISCUSSION

Illumination of guanine and dye complex solution:

The content of guanine lowered with increased time of irradiation. Dark controls, on the other hand, showed a little changes in comparison with the irradiated samples (Table 2, Figure 1).

Table 2. Loss percentages of absorbance in guanine and methylene blue mixture solution(The first group of reaction mixtures) at 274 nm.

Time (Min.)	Illuminated	Dark
30	22.5	6.5
60	24.9	7.1
90	33.3	6.9
120	40.0	7.4
150	46.5	7.4
180	53.7	9.3

* Calculated by excluding the absorbancy of methylene blue.

As shown in above table the loss percentage of guanine was 22.5, 24.9, 33.3,

Fig. 1. Decreasing rate of O.D. in the mixed solution of guanine and dye(the 1st group) by illumination.

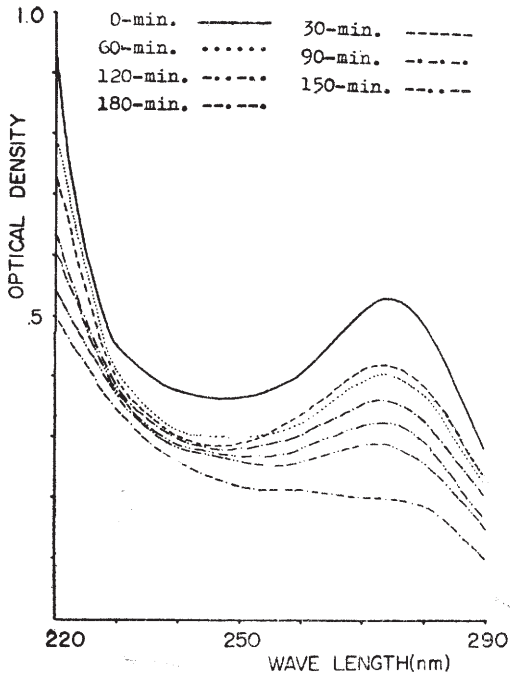
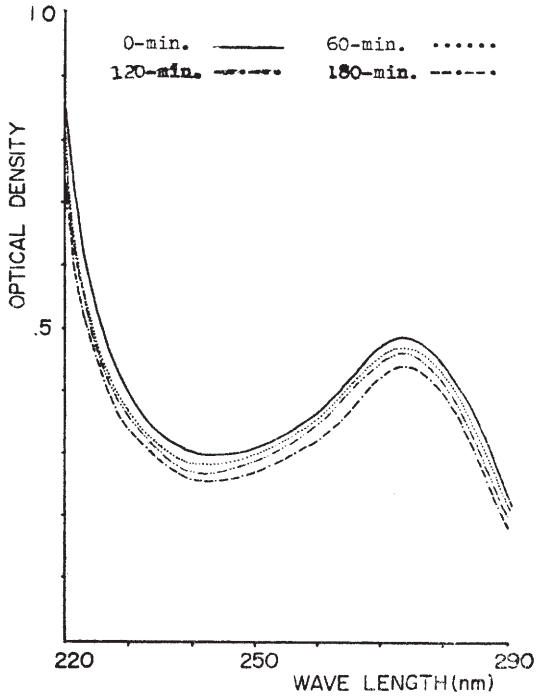


Fig. 2. Decreasing rate of O.D. in the guanine only solution(the 2nd group) by illumination.



40.0, 46.5, and 53.7% for the illuminated, 30-, 60-, 90-, 120-, 150-, and 180-min. illuminated samples, respectively.

Illumination of guanine solution:

Illuminated and nonilluminated guanine control, those are, the second group of reaction mixtures, showed a little decreasing tendency in course of time(Figure 2). The preparation of this guanine control was to obtain the rate of guanine photodecomposition without dye (Table 3).

Illumination of dye solution:

To calibrate the guanine content as mentioned above, methylene blue solution(the third group) was irradiated and its optical density was measured at ultraviolet range. Absorption spectrum of methylene blue at visible range was measured to know the decreasing rate of methylene blue which might be affected by the existence of guanine (Table 4, Figure 3, 4, 5, and 6).

The loss percentages of absorbance of guanine and methylene blue mixture from 600 to 700nm, which is absorption range of methylene blue, were 48.1, 67.4, and 85.4% for the illuminated time 60-, 120-,

Table 3. Loss percentages of absorbance in guanine only solution(the second group of reaction mixtures) at 274nm.

Time (Min.)	Illuminated	Dark
60	2.1	0
120	9.1	1.2
180	8.1	3.9

and 180 minutes. For the dark control the rates were 25.3, 36.4, and 46.2%, respectively. And so, the net loss percentages of absorbance of methylene blue which catalyzed by light were 22.8, 31.0, and 39.2%.

Table 4. Loss percentages of the first group of reaction mixtures (guanine and methylene blue mixture) and the third group of reaction mixtures(methylene blue solution) at 668nm.

Time (Min.)	The first reaction mixture at 668nm.		The third reaction mixture at 668nm.	
	Illuminated	Dark	Illuminated	Dark
30	26.4	13.3	54.3	—
60	48.1	25.3	71.4	25.5
90	58.7	30.4	91.1	—
120	67.4	36.4	81.7	37.9
150	77.2	41.8	95.0	—
180	85.4	46.2	96.9	48.4

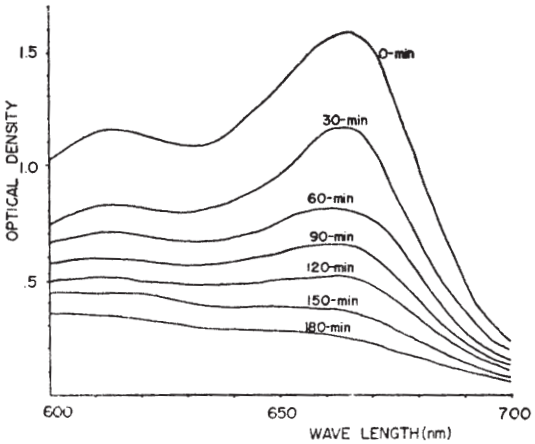


Fig. 3. Decreasing rate of O.D of methylene blue in the guanine and methylene blue mixture solution(the 1st group) by illumination.

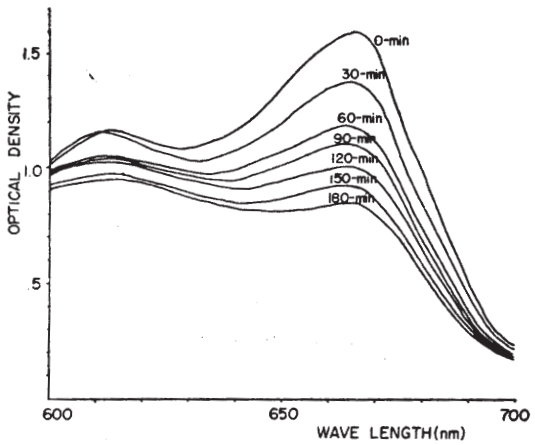


Fig. 4. Decreasing rate of O.D of methylene blue in the guanine and methylene blue mixture solution(the 1st group) in dark.

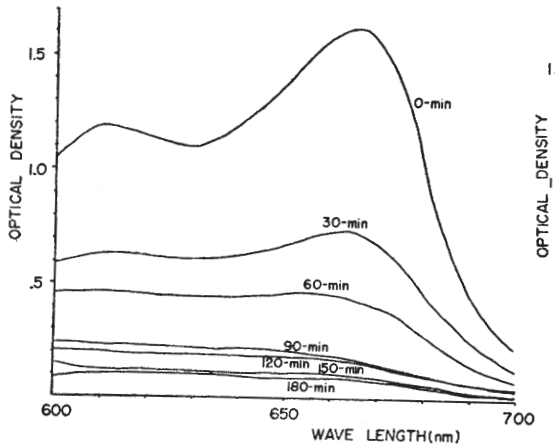


Fig. 5. Decreasing rate of O.D in the methylene blue only solution(the 3rd group) by illumination.

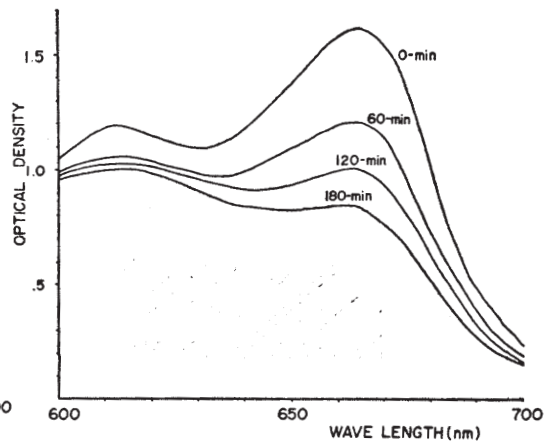


Fig. 6. Decreasing rate of O.D in the methylene blue only solution(the 3rd group) in dark.

In dye only solution, loss percentages were 71.4, 81.7, and 96.9% for 60-, 120-, and 180-minutes and for the dark control, 25.5, 44.7, and 48.4%. In this case, the net loss percentages of absorbance of methylene blue catalyzed by light were 45.9, 43.8, and 48.5%.

Consequently the methylene blue in methylene blue only solution was photodecomposed very rapidly than in guanine mixed solution. It is obvious that methylene blue molecule accelerate the photodecomposition rate of guanine. Decomposition of methylene blue, on the other hand, was interfered with existence of guanine. The methylene blue molecule might be masked by guanine molecule in some site or the guanine solution might act as buffer solution of itself.

Ponce-De León and Cabrera-Juàrez(1970) reported that guanine residue of DNA was decreased fastly in acidic *p*H than in alkaline *p*H. Singer and Fraenkel-Conrat(1966) found that alkaline *p*H had been more effectable on guanine residue of TMV-RNA with proflavine and thiopyronine than neutral *p*H. The present results agree with the latter than the former. It is not clear, however, that photodecomposition mechanism of guanine residues in DNA or RNA could be matched with photodecomposition mechanism of guanine base.

摘 要

Methylene blue의 촉매에 의해 guanine의 광분해 현상은 크게 증가되었다. 색소가 첨가되지 않았던 시험관 내에서의 guanine의 감소비율은 시간 60, 90, 180분에 따라 2.1, 9.1, 8.1% 였음에 비해 색소첨가 구에서는 24.0, 40.0, 53.7%로 훨씬 높은 수치를 나타낸다. 한편 methylend blue 자체의 광조사에 따른 탈색 현상은 guanin이 첨가된 반응액내에서 오히려 적은 수치를 나타냈다.

시간 30, 60, 90, 120, 150, 180분에 따른 methylene blue 단독 용액의 흡광도 감소비율은 54.0, 71.4, 81.1, 81.7, 95.0, 96.9% 였음에 비해 guanine과 공존했던 용액의 흡광도 감소비율은 26.4, 48.1, 58.7, 67.4, 77.2, 85.4% 였다. 이들 각각의 dark control을 제한 값 즉 광조사에 의하지 않고 자체 NaOH 용액의 영향으로만 탈색된 비율은 시간 60, 120, 180분에 따라 guanine과 색소의 공존 시험관에서는 25.3, 36.4, 46.2%, 색소 단독 존재구에서는 25.5, 37.9, 48.4%의 수치를 각각 나타냈는데 광조사에 의해 감소된 량에서 dark control을 제한 값은 시간 60, 120, 180분에 따라 공존시험구에서는 22.8, 31.0, 39.2% 였고 색소단독 존재구에서는 45.9, 43.8, 48.5% 였다.

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