PHOTODYNAMIC ACTION OF LUMIFLAVIN ON THE TEMPLATE DNA OF RNA POLYMERASE

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1. Introduction

A number of reports concerning the interactions between nucleic acids or enzymes and dyes were presented. Among them, the photodynamic action of riboflavin has been shown to lead the interaction of enzymes [1] and bacteriophage [2] or the mutation of *E. coli* [3,4]. Bacteriostatic actions of riboflavin on some organisms have been observed in light [5]. However, few studies on the action of flavin on template DNA and DNA-dependent RNA polymerase (ribonucleoside triphosphate, RNA nucleotidyl transferase, EC 2.7.7.6) were presented. In this report, data will be presented which show the photodynamic action of lumiflavin on the template DNA of RNA polymerase and its specific action to guanine bases in the DNA molecules.

2. Materials and methods

Calf thymus DNA was purchased from Sigma Chemical Co. (St. Louis) or from Worthington Biochemical Co. (Freehold, N. J.). *Micrococcus lysodeikticus* DNA was the product of Miles Laboratories, Inc. (Elkhart, Indiana). The DNAs were further purified by phenol extraction. Lumiflavin was the gift from the Institute of Tokyo Tanabe Pharmaceutical Co. (Tokyo) and purified by the recrystallization from acetic acid before use.

In experiments on photodynamic action, 1 ml of 10 mM Tris–HCl, pH 7.5, containing 1 mg DNA, 10 mM KCl and lumiflavin as required was placed in 2 cm petri dish and irradiated with 200 W photoflood lamp (Toshiba Ref-lamp RS) at a distance of 15 cm. The petri dishes were covered with a filter (Toshiba photographic plate glass) removing the light below 300 nm and cooled in ice during irradiation. It was about 6×10^4 lux at the surface of the DNA solution behind the filter. At selected intervals, 20 μl aliquots were removed and the template activities of RNA polymerase were measured. The base composition of DNA was determined by the method of Wyatt [6]. The melting temperature of DNA was measured by Hitachi Model 124 Recording Spectrophotometer equipped with Cell Cooler, Model SPD-H124 and Temperature Programmer, Model KPC-3 by Komatsu Electronics Inc..

The holoenzyme of DNA-dependent RNA polymerase was extracted from *E. coli* K-12 and purified and assayed according to the methods of Burgess [7,8].

3. Results

The experiments of photosensitized inactivation were carried out with two DNAs, calf thymus DNA and *M. lysodeikticus* DNA, which have a considerable difference in GC contents. As it is shown in fig.1, the irradiation of the DNAs in the presence of lumiflavin resulted in the rapid decrease of the template activities. The rates of the photoinactivation of calf thymus DNA were larger than those of *M. lysodeikticus* DNA in all cases measured by the incorporation of [^{14}C]AMP or [^{14}C]CMP or [^{14}C]GMP. [^{14}C]AMP incorporation was reduced maximally with the calf thymus DNA template after its irradiation in the presence of the dye, while the minimum decrease of the incorporation of the labeled compound was observed, when *M. lysodeikticus* DNA irradiated in the same conditions was used as a template.

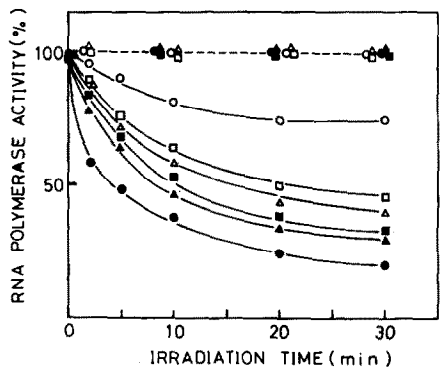

Fig.1. Template activity of irradiated DNA in the presence of lumiflavin on RNA synthesis. Irradiation condition of DNA in the presence (——) and absence (– – –) of 0.01 mM lumiflavin was described in Materials and methods. RNA polymerase activities were assayed by the incorporation of [8-^{14}C]AMP (–●–), [8-^{14}C]GMP (–■–) and [U-^{14}C]CMP (–▲–) on calf thymus DNA and [8-^{14}C]AMP (–○–), [8-^{14}C]GMP (–□–) and [U-^{14}C]CMP (–△–) on *M. lysodeikticus* DNA. The standard activities of RNA polymerase on calf thymus DNA template measured by incorporation of [8-^{14}C]AMP, [8-^{14}C]GMP and [U-^{14}C]CMP were 1.8 nmol, 2.1 nmol and 2.0 nmol according to Burgess respectively. For the other template, *M. lysodeikticus* DNA, the activities measured by [8-^{14}C]AMP, [8-^{14}C]GMP and [U-^{14}C]-CMP were 0.2 nmol, 1.2 nmol and 0.6 nmol. The ordinate indicates the remaining activity of RNA polymerase as percentage of the original activity.

The base specificity of photodynamic action of lumiflavin was studied by analyzing the changes between the base quantities of DNA before and after the irradiation. The results in table 1 reveal that the quantity of guanine bases alone decreases after the irradiation of the DNA in the presence of lumiflavin, and that the photodynamic action of lumiflavin on template DNA is specific to guanine bases.

In other experiments, the actions of some flavin derivatives were compared (table 2), and it was found that the lumiflavin was the most effective one to inactivate the template DNA. The effects decreased with increasing the size of side chain from lumiflavin to FAD. However, lumichrome which consists of alloxazine and methyl groups was much less effective than the isoalloxazine derivatives were.

To estimate the GC content remaining after the irradiation of DNA, the measurements of the melting temperature of the irradiated DNA were carried out for every irradiation time. As shown in table 3, the rate of decrease of the GC content compared to the quantity of total bases in either DNA was almost the same, while the rate compared to the content of GC pairs which were present before irradiation (shown by the values in parentheses) was considerably different in both DNAs.

Table 1
Base compositions of irradiated DNA in the presence and absence of lumiflavin

	Base composition (mole%)		
	Native DNA	Irradiated DNA	
		- lumiflavin	+ lumiflavin
Adenine	29.5	29.6	29.1
Guanine	22.7	21.7	18.2
Cytosine	21.2	21.2	21.2
Thymine	26.8	27.4	26.8

The DNA solutions in the presence and absence of 0.01 mM lumiflavin were irradiated for 30 min. Other irradiation conditions were as described in Materials and methods. Irradiated DNA solutions were dialyzed against 10 mM Tris–HCl, pH 7.5 and hydrolyzed with 60% $HClO_4$ by the method of Wyatt. Base compositions of the irradiated DNA were expressed by mole% adopting 21.2% as the normal value of cytosine. Values are the means of three experiments.

Table 2
Effect of flavins on photosensitized inactivation of DNA-dependent RNA polymerase activity

	Concentration (mM)	RNA polymerase activity (%)
None	–	100
Lumiflavin	0.01	29
Riboflavin	0.01	52
FMN	0.01	96
FAD	0.01	100
FAD	0.5	50
Lumichrome	0.1	86
Lumichrome	1.0	54

The mixtures containing 1 mg calf thymus DNA per ml of 10 mM Tris–HCl, pH 7.5 and indicated concentration of flavins were irradiated for 15 min and assayed as described in Materials and methods. RNA polymerase activity was expressed as percentage of that observed in the absence of flavins.

Table 3
Melting temperatures of the irradiated DNA in the presence of lumiflavin

Irradiation time (min)	Calf thymus DNA		*M. lysodeikticus* DNA	
	T_m(°C)[a]	GC(%)[b]	T_m(°C)[c]	GC(%)[d]
0	84.4	36.8(100.0)	82.4	73.2(100.0)
15	81.7	30.2(82.0)	79.6	67.6(92.5)
30	80.8	28.0(76.5)	78.9	66.2(90.5)
60	79.6	25.1(68.2)	77.5	63.4(86.5)

[a] 20 μg of DNA per ml of standard saline–citrate solution (0.15 M NaCl plus 0.015 M Na-citrate, pH 7.0) was used.
[b] Calculated from the equation by Marmur and Doty [11], GC(%) = 2.44(T_m, 69.3°C).
[c] 25 μg of *M. lysodeikticus* DNA per ml of 0.01 M Tris–HCl, pH 8.0, 0.01 M NaCl was used.
[d] Calculated from the equation by Owen et al. [12], GC(%)[tan(70.077 + 3.32 log[Na^+])](T_m, 175.95°C) + 260.34.

4. Discussion

In the above experiments, the more remarkable photosensitized inactivation of template DNA was shown when the labeled substrate, base of which is present at higher ratio in the template DNA, was used for the assay with RNA polymerase. On the other hand, it was also shown that the photodynamic action of lumiflavin was specific to guanine bases of the DNA template (table 1), although the mechanism had not been completely elucidated. However, the inactivation rate of the calf thymus DNA was larger than that of *M. lysodeikticus* DNA (fig.1) despite the lower GC content of the former.

The results may be explained, first, with the estimation that the ratios of guanine bases modified or decomposed to the total guanine bases in calf thymus DNA are larger than those in *M. lysodeikticus* DNA, which were calculated from the values of GC contents of both DNAs (table 3). Namely, the remarkable and rapid decrease of the GC content of calf thymus DNA may exert an influence on the conformation of DNA and result in the reduction of the nature as a double-stranded DNA on which the activity of the holoenzyme of RNA polymerase is dependent.

Secondly, the results may be caused from the differences in the distribution of damaged guanine bases between both template DNA molecules. In this connection, the differences in the number of the initiation sites of RNA synthesis between both template DNAs may also result in the differences in the rates of the template DNA inactivation by the photosensitization, because the number and lengths of RNA molecules synthesized will be dependent on the number of initiation sites on the DNA molecule, and accordingly varied with the number and distribution of damaged guanine bases in DNA.

As shown in table 2, the most effectiveness of photosensitized action of lumiflavin suggests the possibility that the mechanisms of the action involve the specific and direct interactions between the flavin and the guanine bases in DNA molecule which were unable to find between the other isoalloxazine derivatives and the guanine bases [9]. The results in table 2 also suggest the flavin may be more effective in the photosensitized inactivation of nucleic acid than the dye such as methylene blue as it was reported by Tsugita et al. [10] that riboflavin was more effective in the inactivation of tabacco mosaic virus RNA than methylene blue.

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