REACTIONS OF METHYLGLYOXAL WITH NUCLEIC ACIDS

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1. Introduction

Stachelin [1, 2] observed that glyoxal and other 1,2-dicarbonylic compounds react with the guanine residues of tobacco mosaic virus RNA. Nakaya et al. [3] and Shapiro et al. [4] demonstrated that glyoxal not only reacts with guanine but also, to a lesser degree, with the other nucleic acid bases. Staehelin [1] and Shapiro et al. [5, 6] elucidated the structure of the adducts formed between 1,2-dicarbonylic compounds and guanine and its derivatives. The reactions of glyoxal and kethoxal (3-ethoxy-2-ketobutyraldehyde) with RNA [3, 4, 7–11], and of glyoxal with DNA [3, 12] have also been studied. Recently, Broude and Budowsky [13] studied the kinetics of the reaction of glyoxal with the component bases of nucleic acids.

Differences have been seen in the properties of the different 1,2-dicarbonylic compounds. Shapiro et al. [4] observed that under certain conditions glyoxal reacts with the guanine, cytosine, and adenine residues of RNA, while kethoxal reacts only with guanine. Litt [10] observed that although both glyoxal and kethoxal react with the yeast phenylalanine tRNA, kethoxal inactivates its phenylalanine acceptor activity much more than glyoxal does.

Klamerth [14], studying the mode of action of glyoxal in fibroblasts, and Zwaig and Diéguez [15] and Krymkiewicz et al. [16], studying the mode of action of methylglyoxal (MG) in bacteria, demonstrated a lethal effect associated with an inhibition of the synthesis of proteins, RNA and DNA. It has been demonstrated [17–20] that MG is a metabolic intermediary in bacteria.

This paper reports studies of the reactions of MG with RNA and DNA. The results show that [^{14}C]methylglyoxal, [^{14}C]MG, binds to tRNA and DNA. A much greater quantity of [^{14}C]MG binds to heat-denatured DNA than to native DNA. The quantities of [^{14}C]MG that bind to polyadenilic, and polycytodylic acids are 7% and 3%, respectively, of the quantity that binds to polyguanylic acid. [^{14}C]MG does not bind to polyuridylic acid. Tris(hydroxymethyl)aminomethane reacts with MG and strips it off tRNA. The rate of reaction of MG with tRNA and heat-denatured DNA increases with pH. The tRNA-MG adduct is stable at pH 11, while the heat-denatured DNA-MG adduct undergoes a slow decomposition above pH 8.

2. Materials and methods

[U-^{14}C]glycerol (specific radioactivity, 13.3 Ci/M) was obtained from The Radiochemical Centre (Amersham/Searle Corp.).

Yeast tRNA, calf thymus DNA, and synthetic polynucleotides were obtained from Sigma Chemical Co.

All the other chemicals employed were of the highest purity commercially available.

Native calf thymus DNA was heated at 100° for 15 min and then cooled rapidly in ice to obtain heat-denatured DNA [3].

[^{14}C]MG was produced by a mutant of *E. coli* [15, 21, 22], as previously described [16]. A 5 mM glycerol concentration containing 0.3 μCi/ml [^{14}C]glycerol was used. The [^{14}C]MG was purified as previously described [16], put in a Dowex-1 (Cl^-) column (1 × 15 cm) and then eluted with water. The eluted material was concentrated in a rotatory evapor-

ator and used to study the reactions of MG with the nucleic acids. Although the absorption spectrum (200–700 nm) of the material used indicated the presence of an impurity, the passage of a sample of the material through a Sephadex G-10 column (0.9 × 52 cm) permitted the isolation of the impurity which proved not to be radioactive [23].

The MG was measured colorimetrically after reacting with 2,4-dinitrophenylhydrazine by Wells's method [24], as modified by Cooper and Anderson [19].

The [^{14}C]MG bound to nucleic acids was measured in duplicate reaction mixtures using the Millipore filter method described by Litt and Hancock [8]. 10 μl samples of the reaction mixtures were added to 1 ml of ice-cold water and precipitated with 1 ml of cold 10% trichloroacetic acid. In 72% of the cases, the deviation between the measurements of the duplicate samples was less than 10%. In all cases the values used represent the average of the two measurements.

Dried filters were immersed in 2 ml of scintillation fluid (4 g/ℓ PPO–50 mg/ℓ POPOP in toluene) and counted in an automatic scintillation spectrometer (Model 720, Nuclear-Chicago Corp.) to 3% statistical error at 68% probability. Counting efficiency, determined by internal standarization, was 75%.

3. Results and discussion

An effort was made to determine if the radioactivity of the reaction product formed in the incubation of tRNA with the [^{14}C]MG preparation was due to the reaction of the tRNA with the [^{14}C]MG itself or with some radioactive impurity present in the preparation. Since, at pH 11, MG is transformed into lactic acid [13] and tRNA-MG adduct is stable (see below), the following experiment was performed. Tubes containing the [^{14}C]MG preparation were incubated at pH 11 and 37°. At intervals the quantity of untransformed MG was measured, tRNA was added, left for 90 min, precipitated, and then the radioactivity of the precipitated tRNA was measured. Table 1 shows that the radioactivity was proportional to the quantity of untransformed MG present in the medium.

The kinetics of the reaction between MG and the nucleic acids were studied. Between 0.5 and 5 mM of MG the tRNA-MG adduct formed after 1 hr of reaction

Table 1
The binding of [^{14}C]methylglyoxal, preincubated in alkaline medium, to tRNA.

Time (min)	MG concentration (%)	Radioactivity bound to tRNA (%)
0	100	100
10	47	48
20	25	26
60	7	10

Reaction mixtures of 1 ml containing 0.1 M sodium bicarbonate pH 11 and 0.84 mM [^{14}C]MG were incubated at 37°. At different times 0.1 ml samples were taken to measure the MG concentration, 5 mg tRNA was added to the reaction mixture which was incubated for 1 hr and then the quantity of radioactivity bound to the tRNA was measured.

was proportional to the concentration of MG. Fig. 1 shows the time course of the reactions, at pH 7.5 and 37°, of MG with tRNA, native DNA, and heat-denatured DNA. It can be seen that the reaction of MG with tRNA reaches its equilibrium in 1 hr. It can be observed as well that a much greater quantity of MG is bound to heat-denatured DNA than to native DNA. This result suggests that the structure of DNA protects the guanine residues from the attack of MG. The small reaction of MG with native DNA could be

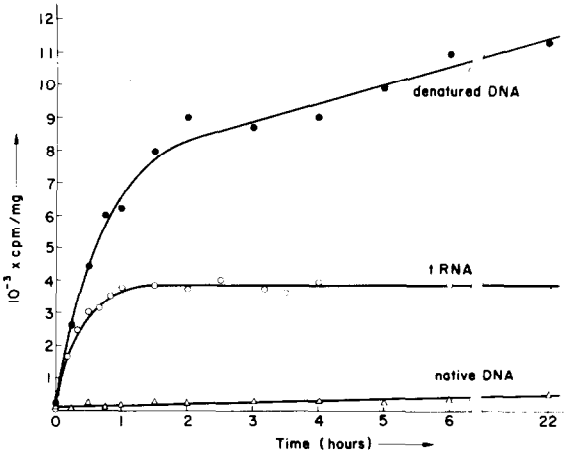

Fig. 1. The binding of [^{14}C]MG to nucleic acids at pH 7.5 and 37°. The reaction mixtures (0.4 ml final volume) contained 10 mM sodium phosphate pH 7.5, 1.2 mM [^{14}C]MG, and 2 mg tRNA or 2 mg DNA or 2 mg heat-denatured DNA.

Table 2
The binding of [^{14}C]methylglyoxal to synthetic polynucleotides.

Time (hr)	Poly-G (cpm/mg)	Poly-A (cpm/mg)	Poly-C (cpm/mg)	Poly-U (cpm/mg)
0.5	5581	582	296	0
1.5	10103	751	322	0
3.0	13067	848	283	0
23.0	12054	824	180	0

Reaction mixtures of 0.24 ml containing 10 mM sodium phosphate pH 7.5, 2 mg of one of the synthetic polynucleotides, and 1.2 mM [^{14}C]MG were incubated at 37°.

due to temporary local openings in double-stranded DNA. Hydrogen exchange studies performed by Printz and Hippel [25] suggest that there are frequent openings and closings of the double-stranded DNA structure at the level of any particular nucleotide pair. Nakaya et al. [3] studied the reaction of DNA and glyoxal, measuring the drop in optical density produced at 269 nm when glyoxal reacts with guanine-containing compounds. They concluded that glyoxal reacts with denatured DNA but not with native DNA. This last conclusion differs with the results obtained here in

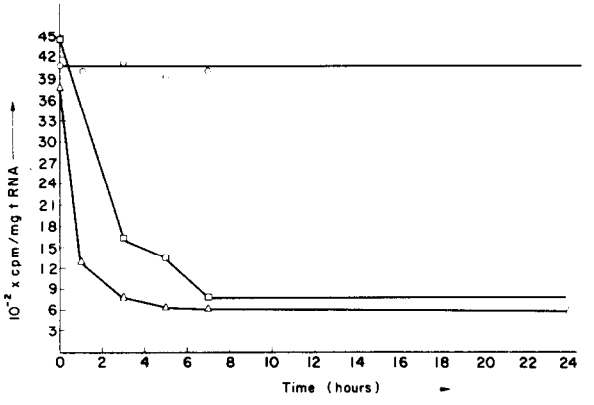

Fig. 2. The effect of tris(hydroxymethyl)aminomethane (Tris) on tRNA-MG adduct. Reaction mixtures of 0.12 ml containing 40 mM sodium phosphate pH 7, 0.5 mg tRNA, and 1.2 mM [^{14}C]MG were incubated for 90 min at 37°. At 0 time 0.12 ml 0.2 M sodium phosphate pH 7 (○—○—○), 0.12 ml 0.2 M Tris pH 7 (△—△—△), or 0.12 ml 0.2 M Tris pH 7 plus 0.16 M sodium borate pH 7 (□—□—□) were added and the quantities of [^{14}C]MG bound to tRNA were measured at different times.

Table 3
The binding of [^{14}C]methylglyoxal to nucleic acids different pH's.

pH	(cpm/mg tRNA)			(cpm/mg heat-denatured DNA)		
	5 sec	1.5 hr	21 hr	5 sec	1.5 hr	21 hr
4	0	1222	6812	0	884	4810
5	0	1456	5798	0	1170	8580
6	0	2730	3666	0	5872	12220
7	40	4420	4830	70	12663	13260
8	104	4030	4628	676	14430	8320
9	2184	7072	9958	5902	13676	9048
10	7800	10400	10712	5330	14456	8138
11	10972	10520	10530	7150	10166	7982

The reaction mixtures (0.12 ml final volume) contained 1.2 mM [^{14}C]MG, 0.5 mg tRNA or 0.5 mg heat-denatured DNA, and 80 mM sodium acetate (pH: 4, 5, 6) or 80 mM sodium phosphate (pH: 7, 8) or 80 mM sodium bicarbonate (pH: 9, 10, 11). Samples were taken to measure the [^{14}C]MG bound to the nucleic acids after 5 sec of reaction at 20° and then the reaction mixtures were incubated at 37°.

the reaction of MG with native DNA and could be due to less sensitivity in the method used by Nakaya et al. [3].

In order to determine the degree of reaction of MG with the different component bases of the nucleic acids, the binding of [^{14}C]MG to different synthetic polynucleotides was studied. Table 2 shows that [^{14}C]MG bound to poly-G to a much greater extent than to the other polynucleotides. After 90 min of reaction the extent of the binding of [^{14}C]MG to poly-A, and poly-C was 7% and 3%, respectively, of its binding to poly-G. No binding to poly-U was seen.

Since tris(hydroxymethyl)aminomethane (Tris) reacts with MG and prevents its inhibition of the synthesis of proteins, RNA and DNA in bacteria [16], the effect of Tris on the tRNA-MG adduct was studied. Fig. 2 shows that the Tris stripped the MG off the tRNA. A similar effect of Tris on the poly-G-MG adduct was observed. The studies of Litt and Hancock [8] and Litt [9] report the decomposition of the tRNA-kethoxal adduct in a medium containing 0.1 M Tris pH 7.8. The adduct's decomposition was interpreted as a result of the alkalinity of the medium. The results presented here suggest, rather, that it was due to the effect of the Tris.

Previous studies [8, 13] show a stabilizing effect

of borate on the adducts formed by guanine derivatives with 1,2-dicarbonylic compounds. Consequently the effect of 0.1 M Tris on the tRNA-MG adduct in presence of sodium borate was studied. In fig. 2 it can be seen that an 80 mM sodium borate concentration did not inhibit the decomposition of the tRNA-MG adduct by Tris.

A study was made of the reaction of MG with tRNA and denatured DNA at different pH. The results in table 3 showing the measurements made after 5 sec of reaction demonstrate that the rate of the reactions increased with the increase in pH. After 21 hr of incubation the quantity of MG bound to the tRNA in acid and basic media was greater than that bound to the tRNA in the neutral medium (table 3). This could be due to a greater quantity of tRNA guanine residues being made accessible to the MG by the denaturing of tRNA in acid or basic media. The fact that the changes in pH did not have a similar effect on denatured DNA, whose structure had been previously disorganized by heat, agrees with this supposition. It is also observed (table 3) that the tRNA-MG adduct is stable at pH 11, while the heat-denatured DNA-MG adduct undergoes a slow decomposition above pH 8.

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