EFFECT OF FORMALDEHYDE AND ITS AMINOMETHYLOL DERIVATIVES ON STRAINS OF *Escherichia coli* WITH VARIOUS DEFECTS OF DNA REPAIR SYSTEMS

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Aminomethylol compounds formed by the reaction between formaldehyde and amino acids, like formaldehyde itself, have a marked lethal action on strains of *Escherichia coli* with various defects of DNA repair systems. Correlation was found between the degree of depurinization of DNA caused by different aminomethylol derivatives *in vitro* and the inactivating action of these derivatives on bacteria. It is suggested on the basis of the results that the inactivating effect of formaldehyde and its aminomethylol derivatives is evidently due to the formation of depurinized regions in the bacterial DNA rather than to dimerization of the purine bases.

KEY WORDS: survival; depurinization; monomethylolglycine; monomethylollysine; formaldehyde.

Despite the extensive use of formaldehyde (FA) as an inactivator, the mechanism of its inactivating action on the cell is still only little understood. We know that FA reacts actively with the amino groups of proteins and amino acids [3] and much more slowly (with a reaction velocity 1000 times lower) with free amino groups of DNA bases [10]. In experiments *in vitro* the writers showed [4] that FA, bound primarily in the cell with free amino acids and proteins, in the form of aminomethylol compounds, can react independently with nucleotides in DNA. As a result of this reaction destabilization of the N-glycoside bond in deoxyadenosine or deoxyadenosine-5-phosphate takes place, and its action on DNA causes removal of adenine from the nucleic acid [5, 6]. Under these circumstances the degree of destablization of the N-glycoside bond in deoxyribosyl derivatives of adenine under the influence of monomethylolglycine (MMG) (a product of the binding of FA with glycine) is much greater than that produced by the action of aminomethylol derivatives of other amino acids, such as monomethylollysine (MML) (a product of the reaction between FA and lysine) [5, 6]. For example, whereas 0.2 M MMG at pH 6.0 and 48°C removes 23 adenine molecules in 1 h from a molecule of single-stranded DNA with a molecular weight of $3 \cdot 10^6$ daltons, 0.2 M MML, under the same conditions, removes only two bases from the DNA molecule [5, 6].

In the investigation described below the action of MMG and MML on bacteria was compared by means of the survival test in order to analyze the role of depurinization of DNA as the cause of the lethal effect of FA. The intention was to obtain differences in the inactivating action of MMG and MML, which differ from each other in the degree of cleavage of the N-glycoside bond in deoxyadenosine. For this purpose strains of *Escherichia coli* with different defects in the DNA repair system were used: Depending on the degree of involvement of these systems in the repair of injuries induced in DNA by FA and its derivatives the mechanism of the lethal action could be judged.

EXPERIMENTAL METHOD

A Soviet preparation of chemically pure [grade "kh.ch."] lysine monohydrochloride was used and glycine was obtained from Serva (West Germany). A pharmaceutical preparation of formalin was neutralized as described previously [7] and the concentration of FA in it was determined by an iodometric method [2]. Different concentrations of pure FA and also FA in the presence of 0.1 M solutions of glycine and lysine [4] were used in the experiments.

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TABLE 1. Inactivating Action of FA and its Amino-Derivatives on Isogenous Strains of *E. coli* K-12 and *E. coli* B (M ± m)

Concentration of compound	Compound studied	Number of cells surviving after treatment of strains, %								
		KS112 uvr+	KS113 uvr A6	KS114 uvr E	KS 120 pol A+	KS 163 pol. AI	DM 843 wild-type	DM 844, lex AI	WP-2 wild-type	WP-3 rec A
$1 \cdot 10^{-2}$	MMG	38±6,4	3,9±2,3	31±6,2	12±5,0	—	24±4,8	—	31±3,2	0,4±0,3
	MML	42±6,0	9,9±3,3	33±5,1	17±4,6		40±4,6		48±4,0	2,2±0,8
	FA	57±5,3	6,9±2,9	34±6,0	21±4,2		39±5,6		51±4,4	1,8±0,7
$5 \cdot 10^{-3}$	MMG	47±5,7	9,9±3,3	32±5,0	25±6,2	—	30±4,6	13±4,8	44±4,0	4,0±1,0
	MML	56±5,3	14±4,2	41±5,6	42±4,4		48±4,1	25±4,5	56±3,5	7,5±1,7
	FA	58±5,2	17±4,6	43±5,7	36±4,8		48±4,1	18±4,5	60±3,4	7,1±1,4
$2,5 \cdot 10^{-3}$	MMG	55±3,3	19±4,8	42±4,6	50±7,1	—	46±4,5	17±5,3	53±3,6	12±1,8
	MML	64±4,8	24±5,4	42±5,6	61±4,4		57±4,0	38±4,4	68±3,1	22±2,4
	FA	60±6,3	33±6,3	55±7,1	54±4,3		58±3,6	24±3,9	63±3,2	22±2,4
$1,25 \cdot 10^{-3}$	MMG	62±4,9	26±5,6	52±3,9	63±4,1	2,0±1,6	63±3,6	28±3,4	63±3,9	33±3,0
	MML	76±4,0	28±5,8	71±4,1	70±4,6	8,2±3,5	71±3,2	62±4,0	74±3,3	41±3,3
	FA	83±3,2	56±7,5	65±4,4	69±4,6	7,1±3,0	79±2,7	54±4,0	73±5,3	53±3,6
$0,6 \cdot 10^{-3}$	MMG	—	—	—	—	39±5,3	—	53±3,5	—	—
	MML					41±9,4		84±2,6		
	FA					55±5,0		74±2,9		
$0,3 \cdot 10^{-3}$	MMG	—	—	—	—	67±4,3	—	—	—	—
	MML					81±5,0				
	FA					82±3,3				
$0,15 \cdot 10^{-3}$	MMG	—	—	—	—	82±3,0	—	—	—	—
	MML					83±6,2				
	FA					83±3,2				

The inactivating action of FA and its amino-derivatives was studied on isogenous strains of *E. coli* K-12 and *E. coli* B, of the wild type and with various defects in the DNA repair system: KS 112 uvr^+; KS 113 uvr A6; KS 114 uvr E; KS 120 trp A 58 str^s, pol A^+; KS 163 trp A 58 str^s pol A I; DM 843, F^- lac λ^- str^s; DM 844 F- lac-λ-str^s lex AI; WP = 2 trp^-; WP = 2 trp^- rec A.*

An overnight culture of *E. coli* grown on Difco's Bacto Nutrient Agar was suspended to one-billion concentration in 1/15 M phosphate buffer, pH 6.8, the preparations to be studied were added, and the samples were incubated at 37°C for 30 min. The *E. coli* cells were seeded from $1 \cdot 10^{-5}$ dilutions on dishes with agar medium. The results were read 24-48 h after incubation at 37°C. The number of observations ranged from three to five for each strain.

EXPERIMENTAL RESULTS

The aminomethylol compounds, like FA itself, had a marked lethal action on *E. coli* (Table 1). There were statistically significant differences in the degree of inactivation by MMG and MML. On treatment of all the strains with all the concentrations of preparations MMG had a stronger action on *E. coli* (the differences were most marked when high concentrations were used). This result is in full agreement with those obtained *in vitro*, when differences were found in the degree of destabilization of the N-glycoside bond between the substances studied [5, 6]. The weaker inactivating effect of FA (about equal to the effect of MML) can possibly be explained by differences in the intensity of the action of compounds formed by interaction between FA and intracellular amines on DNA (differences in the degree of destabilization of the N-glycoside bond) [6].

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All strains defective for the DNA repair system used in this investigation possessed increased (compared with the wild type) sensitivity to the action of FA and its derivatives, in full agreement with earlier observations [11-13]. The exception was strain *E. coli* K-12 KS 114, a mutant of uvr E⁻. The sensitivity of this mutant was about equal to that of the wild-type strain *E. coli* K-12. According to Smirnov [8], the uvr E⁻ mutant is just as resistant to γ rays and to MMS. The suggestion [8] seems right that on correction of defects of parental DNA in uv-irradiated bacteria a specific reaction (or reactions) requiring the product of the uvr E gene takes place.

The participation of the same systems in the repair of DNA injuries induced by FA and its derivatives, and also the identical relationship between the sensitivity of the various strains to the action of MMG, MML, and FA (the differences are explained by the degree of depurinization of DNA) could point to a general mechanism of action of these various compounds on bacterial DNA.

It is clear from Table 1 that the product of the uvr-A6 gene, as well as that of the rec A gene, and to an even greater degree, the product of the pol AI gene, participate in the repair of injuries to DNA induced by FA and its derivatives. We know [10] that the uvr A⁻ mutant is lacking in uv-endonuclease, cleaving uv-irradiated DNA, and this has been interpreted [12, 13] as proof of the formation of purine dimers in DNA by analogy with the pyrimidine dimers formed after uv-irradiation. However, during treatment of DNA *in vitro* no purine dimers could be detected [14], nor has the hypothesis that uv-endonuclease repairs DNA containing cross-linkages between its bases that differ from cyclobutane dimers ever been verified experimentally [9]. On the contrary, it has recently been found that uv-endonuclease, besides cleaving uv-irradiated DNA, probably duplicates the activity of endonuclease II [9], an enzyme which participates in the repair of depurinized regions of DNA [15]. Differences in the degree of inactivation of strains defective for DNA-polymerase I and uv-endonuclease (Table 1), in the light of what has just been said, may be regarded as confirmation of the validity of the hypothesis that depurinized regions requiring the presence of DNA-polymerase I for their repair, are formed in bacterial DNA under the influence of FA. Furthermore, the product of the rec A gene also participates in the repair of injuries induced in DNA by FA and aminomethylol derivatives.

Aminomethylol derivatives, like FA itself, thus have a marked lethal action on *E. coli*. Inactivation of the bacteria under these circumstances is evidently determined by the injuries in DNA. MMG has the strongest inactivating effect. The degree of depurinization of DNA *(in vitro)* was shown to depend on the inactivating action of MMG and MML on bacteria. On the basis of these results it can tentatively be suggested that there is a common mechanism for the inactivating action of aminomethylol compounds and of FA on bacteria. The inactivating effect is probably due to the formation of depurinized regions in the bacterial DNA rather than to dimerization of the purine bases [12, 13].

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