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Properties and Mode of Action of a Bactericidal Compound (=Methylglyoxal) Produced by a Mutant of *Escherichia coli*

NORBERTO KRYMKIEWICZ, EUGENIA DIÉGUEZ, USUA D. REKARTE,[1] AND NOÉ ZWAIG

Departamento de Biologia Celular, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 10098, Caracas, Venezuela

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A lethal product (BPG) produced by a glycerol kinase mutant of *Escherichia coli* was purified, and its mode of action on *E. coli* was studied. At concentrations where BPG strongly inhibits in vivo deoxyribonucleic acid, ribonucleic acid, and protein synthesis, it produces small effects on other functions: slight inhibition of respiration and small changes in intracellular pools of substrates, nucleic acids degradation, and adenosine triphosphate levels. BPG also inhibits in vitro protein synthesis and produces inactivation of bacteriophage T4. The bactericidal product has been identified in another laboratory as methylglyoxal (MG). By comparing BPG and MG, we confirmed this observation and concluded that the activity found in our BPG preparation is due to its MG content. We also observed that MG is able to react with guanosine triphosphate. According to these results, it is interpreted that MG could act directly on macromolecular synthesis by reacting with the guanine residues of nucleic acids and its precursors.

Glycerol kinase, the first enzyme in glycerol metabolism of *Escherichia coli*, is feedback regulated by fructose-1,6-diphosphate (15). A mutant that is insensitive to such control was isolated (15). When cells of this strain are grown on a mineral medium plus a carbon source, e.g., succinate, the addition of glycerol stops further growth, and a few hours afterwards no viable cells can be detected by using rich medium plates (17). The compound which produces this bactericidal effect is released into the medium and can be separated from the cells (16). Some characteristic of this bactericidal product (BPG) have been reported in a previous paper (16): it is a small molecule produced by Hfr and F^+ but not by F^- strains; it has lethal activity on gram-positive and gram-negative bacteria; it inhibits in vivo protein synthesis.

Further studies on the mode of action of BPG were performed. While this work was in progress, the bactericidal product was characterized as methylglyoxal (MG) (E. C. C. Lin, *personal communication*: W. B. Freedberg, W. S. Kistler, and E. C. C. Lin, Fed. Proc. **30**:1284. They found that the bactericidal product is a substrate for glyoxalase I and reacts with 2,4-dinitrophenylhydrazine (DNPH) to produce a derivative indistinguishable from the *bis*-DNPH derivative of MG.

MG was found to be produced in *E. coli*, from dihydroxyacetone phosphate (DHAP), initiating a bypass of the glycolytic pathway (3) (Fig. 1).

In this paper, some properties, as well as the mode of action of partially purified BPG, are reported. From these and similar studies carried out with MG, the identification made by Freedberg et al. was confirmed and the results show that all the activity found in the BPG preparation is solely due to its MG content.

MATERIALS AND METHODS

Chemicals. L-[*U*-^{14}C]isoleucine (specific radioactivity, 312 Ci/mole); [*2*-^{14}C]uracil (specific radioactivity, 52.5 Ci/mole); and [*U*-^{14}C]thymidine (specific radioactivity, 520 Ci/mole) were obtained from The Radiochemical Centre (Amersham/Searle Corp.).

MG was obtained from Sigma Chemical Co. and was further purified by passage through a Dowex-1 chloride column.

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

Bacterial strains. *E. coli* K-12 strain 3000 Y-14, Hfr H, lac$^-$, thiamine$^-$ and *E. coli* B/1,5, phage T1-T5-resistant were both provided by S. Luria; *E. coli* K-12

[1] Research Fellow from the Consejo Nacional de Investigaciones Científicas y Tecnológicas, Venezuela.

strain 43 was constitutive for glycerophosphate system (15).

Production and purification of BPG. Strain 43 was grown with shaking at 37 C in mineral medium (MM) (14) plus 0.2% succinate to 4×10^8 cells/ml (exponential phase). Cells were centrifuged at 7,000 × *g* for 10 min and transferred to a medium containing 0.85% NaCl plus 5 mM glycerol. After 3 hr of incubation with shaking at 37 C, the cells were separated by centrifugation at 7,000 × *g* for 10 min. The supernatant fraction was filtered through a membrane filter (0.45-μm pore size, Millipore Corp.), partially dried in a rotary evaporator and then fully dried in a desiccator containing P_2O_5. This dried material was extracted four times with equal volumes of boiling ether (80 ml of ether for each 1,000 ml of supernatant fluid). BPG was then extracted from the ether phase with 2 volumes of water. With this procedure, 30 to 50% of the original activity was obtained.

In all the experiments, BPG concentration is expressed as the dry weight of purified material in 1 ml of solution.

Estimation of MG. MG was measured colorimetrically after reaction with DNPH by the method of Cooper and Anderson (3).

Measurement of BPG activity. To test BPG activity, different dilutions were added to cultures of strain 3000 Y-14 (ca. 10^6 cells/ml) growing at 37 C in MM plus 0.2% glycerol, 0.2% succinate, and 0.02 mg of thiamine per ml. Inhibition was indicated by lack of turbidity after 16 hr of incubation.

Measurement of lethal effect. The lethal effect of BPG, or MG, was estimated by adding different dilutions to cells of strain 3000 Y-14 (6×10^6 cells/ml) suspended in MM. After 3 hr of incubation at 37 C, the viable counts were determined by plating out appropriately diluted samples of the cultures on agar containing 0.17% Antibiotic Medium 3 (Difco).

Uptake and incorporation of labeled compounds. An exponential-phase culture of strain 3000 Y-14 or strain B/1,5 in MM plus 0.2% glucose and 0.02 mg of thiamine per ml was centrifuged at 4,300 × *g* for 15 min and suspended in MM plus 0.5% glucose to obtain a heavy cell suspension which was added to the complete incubation mixture to initiate the experiments.

Incubations with 2×10^9 cells per ml were carried out in a "basic solution": MM plus 0.5% glucose and 0.02 mg of thiamine per ml to which 1.5×10^{-4} M L-[^{12}C]isoleucine, 3.9×10^{-7} M L-[^{14}C]isoleucine, or 6.0×10^{-4} M [^{12}C]uracil; 3.0×10^{-6} M [^{14}C]uracil or 9.0×10^{-4} M [^{12}C]thymidine; and 6.0×10^{-6} M [^{14}C]thymidine were added, according to the experiment. When 2×10^8 cells per ml were used, 1.5×10^{-5} M L-[^{12}C]isoleucine; 3.9×10^{-7} M L-[^{14}C]isoleucine or 6.0×10^{-5} M [^{12}C]uracil; 3.0×10^{-6} M [^{14}C]uracil or 6.0×10^{-5} M [^{12}C]thymidine; and 9.0×10^{-6} M [^{14}C]thymidine were added. A 1.7- or 0.23-mg amount of BPG per ml; 3 mM MG; 0.1 M 2-amino-2(hydroxymethyl)-1,3-propanediol (Tris) *p*H 7.0 and 50 μg of chloramphenicol (CAP) per ml were added when indicated.

Suspensions and liquid culture were incubated with shaking in a water bath at 37 C.

In uptake experiments (2×10^9 cells/ml), samples of 0.2 ml were collected at different times on a membrane filter and washed with 5 ml of MM.

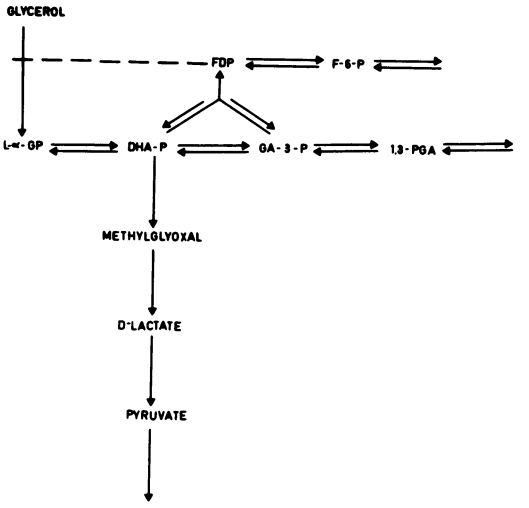

FIG. 1. *Metabolic scheme for methylglyoxal production.*

In incorporation experiments, samples of 0.2 ml (when 2×10^9 cells/ml were used) or 0.5 ml (when 2×10^8 cells/ml were used) were taken at different times and mixed with an equal volume of cold (0 C) 10% trichloroacetic acid. The samples were allowed to stand at 0 C for 30 min, and the acid-insoluble fraction was collected on membrane filters and washed with 5 ml of cold (0 C) 5% trichloroacetic acid.

The filters were dried and placed in scintillation vials to which 5 ml of scintillation fluid [4 g of 2,5-diphenyloxazole and 0.05 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene] was added. The radioactivity was counted in an automatic scintillator-spectrometer (model 720, Nuclear-Chicago Corp.).

Degradation studies. Strain 3000 Y-14 was grown in MM plus 0.2% glucose and 0.02 mg of thiamine per ml into the exponential phase. Incubations with 2×10^9 cells/ml were performed in 2 ml of the basic solution, to which 6.6×10^{-7} M L-[^{14}C]isoleucine or 5.3×10^{-6} M [^{14}C]uracil or 4.0×10^{-7} M [^{14}C]thymidine was added.

After 1 hr of incubation with shaking in a water bath at 37 C, the cells were centrifuged at 4,300 × *g* for 10 min and suspended in 2 ml of the basic solution, to which 1.6×10^{-4} M L-[^{12}C]isoleucine or 1.6×10^{-3} M [^{12}C]uracil or 1.8×10^{-3} M [^{12}C]thymidine was added. Each of these suspensions was equally distributed in two tubes. One contained 1.7 mg of BPG per ml and the other was used as a control. Both were incubated with shaking at 37 C and samples of 0.2 ml were collected at different times and mixed with 0.2 ml of cold (0 C) 10% trichloroacetic acid; the acid-insoluble fraction was treated as indicated above.

In vitro protein synthesis. A cell-free extract from strain 3000 Y-14 was prepared by a modification of the method of Matthaei and Niremberg (11). In the extraction procedure, 0.01 M Tris (*p*H 7.8) was substituted by 0.01 M sodium barbital buffer (*p*H 7.8) The S-30

fraction was used, and its protein content was determined by the method of Lowry et al. (9).

The incubation mixture was prepared as described by Matthaei and Niremberg (11) except that Tris and [^{14}C]valine were replaced by 50 mM sodium barbital buffer (*p*H 7.8) and 5.2×10^{-7} M L-[^{14}C]isoleucine, 4.0×10^{-6} L-[^{12}C]isoleucine. A 1.7-mg amount of BPG per ml and 100 mM Tris (*p*H 7.0) were added when indicated. Total volume of samples was 1.2 ml.

Samples were incubated with shaking in a water bath at 37 C and the proteins were precipitated at different times with an equal volume of 20% trichloroacetic acid containing 0.05 M L-isoleucine.

The acid-insoluble fractions were washed, dissolved in formic acid, and counted in a scintillator-spectrometer as described previously (8).

Estimation of ATP. Cells of strain 3000 Y-14 (2×10^9 cells/ml), obtained from an exponential-phase culture, were incubated in 1 ml of basic solution with the following additions: 1.7 mg of BPG per ml, 2×10^{-3} M 2,4-dinitrophenol (DNP), or 50 μg of CAP per ml, when indicated. After incubation, adenosine triphosphate (ATP) was extracted by a modification of the method of K. L. Field (Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1968). At 30 and 60 min, 0.1-ml samples from the incubation mixture were added to a tube containing 9.9 ml of boiling water. The samples were boiled for 10 min and placed on ice; the ATP content was measured immediately.

The assays of ATP were done in an automatic scintillator-spectrometer by a modification of the method of Cole et al. (2). Dried firefly lanterns (FLE-50, Sigma Chemical Co.) were added to 50 ml of 0.05 M sodium arsenate buffer (*p*H 7.4) plus 0.02 M magnesium sulfate. The extract was centrifuged at 39,000 $\times$ *g* at 4 C for 20 min and the supernatant fraction was kept on ice in the dark until it was used. ATP assays were carried out under red light, in vials of 4 ml. A 0.3-ml amount of 0.05 M sodium arsenate buffer (*p*H 7.4) was added to 1.0 ml of 0.05 M glycine (*p*H 7.4) to which 0.1 ml of centrifuged firefly lantern extract was added and mixed. After this, 0.2 ml of the boiled sample extract was quickly added and mixed, and the vial was put into the scintillator-spectrometer (one channel was set at 1,180 v and the coincidence circuit was switched out). At 20 sec after the addition of the boiled sample extract, the light emitted by the mixture was counted for 1 min. The counts were proportional to the amount of extract added. An ATP standard freshly prepared was assayed at the same time giving a linear relationship between 0.2 and 20 pmoles.

Oxygen uptake. O_2 uptake was measured by a conventional Warburg apparatus. Experiments were performed in a total volume of 1.5 ml. Cells of strain 3000 Y-14 were harvested during exponential growth in MM plus 0.2% glucose and 0.02 mg of thiamine per ml, centrifuged, and concentrated to 2×10^9 cells/ml in basic solution. BPG was added when indicated.

RESULTS

In vivo mode of action of BPG. To correlate the results, the following experiments were carried out under the same conditions: (i) The effect of BPG on the synthesis of macromolecules in intact cells is shown in Fig. 2. The addition of 1.7 mg of BPG per ml inhibits the incorporation of [^{14}C]isoleucine, [^{14}C]uracil, and [^{14}C]thymidine. However, the pattern of inhibition of these compounds is different. The incorporation of [^{14}C]isoleucine is nearly halted from zero time, but that of both [^{14}C]uracil and [^{14}C]thymidine is only completely arrested some minutes later. Under the above conditions, no viable cells were found after 3 hr of treatment.

These differences between protein and nucleic acids synthesis suggested that a lower BPG concentration could produce a selective inhibition of protein synthesis. However, the results with 0.23 mg of BPG per ml ruled out this possibility (Fig. 2).

The effect of BPG on [^{14}C]uracil incorporation in the presence of CAP was studied. The results of Fig. 2 show that ribonucleic acid (RNA) synthesis is halted even in the presence of CAP. This could indicate that the inhibition of protein synthesis is not the cause of the effect of BPG on RNA synthesis. The observed inhibition of RNA synthesis (Fig. 2) is not in agreement with previous results (16), which show a stimulation rather than an inhibition by BPG. This difference can be attributed to the lack of a carbon source in the experiments previously performed. After 30 min of incubation, [^{14}C]uracil incorporation in the presence of a carbon source was estimated to be 13 times higher than in its absence. The fact that stimulation by BPG was observed in former experiments may be ascribed to the presence of a carbon source in the BPG preparation itself. Since in experiments performed in this study the carbon supply was not limiting, RNA synthesis by the controls was high enough to observe clearly the effect of BPG.

As may be seen in Fig. 2, Tris antagonizes the effect of BPG on the synthesis of macromolecules. To determine whether BPG action could be reverted by Tris, this compound was added 12 min later than the inhibitor. As can be observed in Fig. 2, the inhibition caused by BPG could not be reverted by Tris.

(ii) As may be observed in Fig. 3, the inhibition of macromolecular synthesis caused by BPG (Fig. 2) is not related to an impairment of the uptake of radioactive substrates. The pool of [^{14}C]isoleucine, [^{14}C]uracil, and [^{14}C]thymidine is slightly stimulated rather than inhibited in the presence of BPG.

(iii) The inhibition of macromolecular synthesis caused by BPG (Fig. 2) could also be attributed to an enhanced degradation. However, BPG has no effect on protein degradation (Fig. 4) and the small effect observed on RNA and

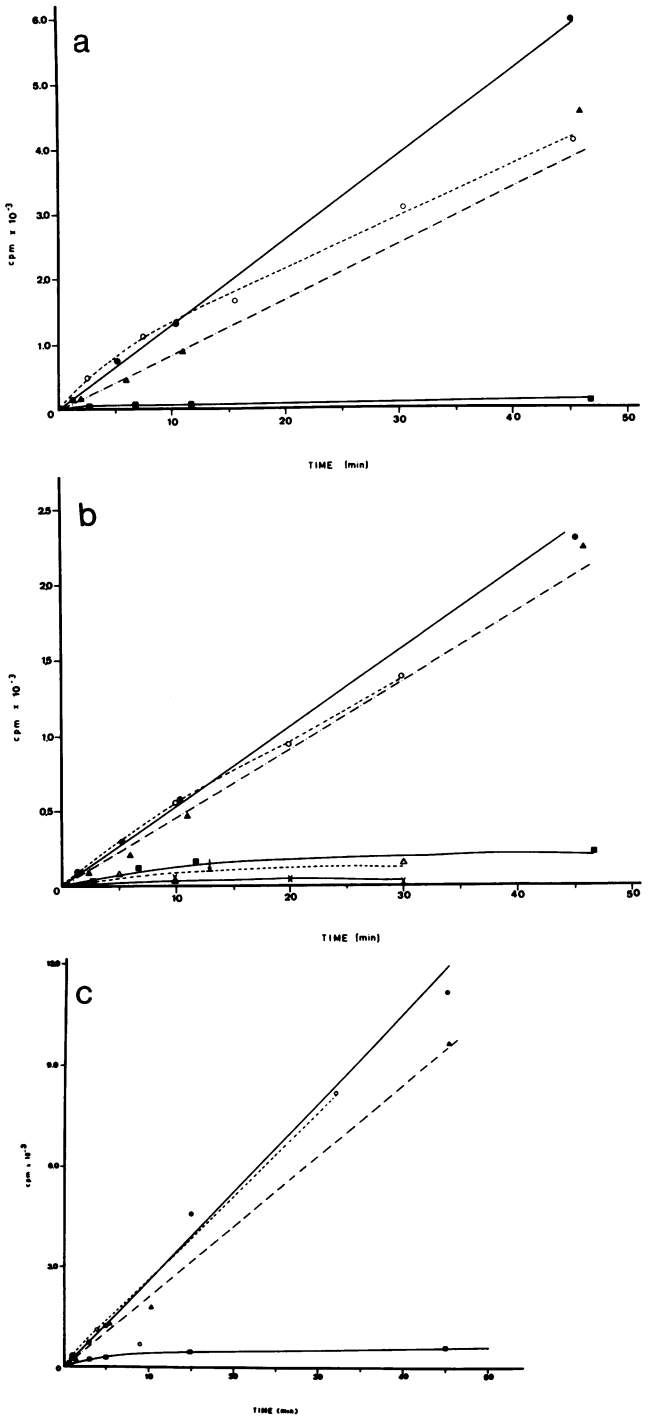

FIG. 2. *Effect of BPG on incorporation of* L-[^{14}C]*-isoleucine* (*a*); [^{14}C]*uracil* (*b*), *and* [^{14}C]*thymidine* (*c*). *Incubations were carried out with* 2×10^9 *cells/ml. Control* (●); *1.7 mg of BPG per ml* (■); *0.23 mg of BPG per ml* (▲); *1.7 mg of BPG per ml* (○) *plus 0.1* M *Tris, pH 7.0, added at zero time; 1.7 mg of BPG per ml* (△) *plus 0.1* M *Tris, pH 7.0, added 12 min after; 1.7 mg of BPG per ml* (×) *plus 50 μg of CAP per ml added at zero time.*

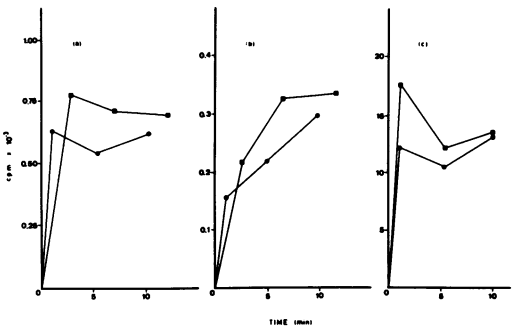

FIG. 3. *Effect of BPG on the pool of:* L-[^{14}C]*isoleucine* (*a*); [^{14}C]*uracil* (*b*) *and* [^{14}C]*thymidine. Incubations were carried out with 2* × *10^{9} cells/ml. Control* (●); *1.7 mg of BPG per ml* (■). *The pool of the radioactive substrates represented here was calculated by substracting the acid-insoluble radioactivity from the total uptake of radioactivity measured as described in Materials and Methods.*

deoxyribonucleic acid (DNA) degradation (Fig. 4) cannot explain the inhibition of [^{14}C]uracil and [^{14}C]thymidine incorporation seen before (Fig. 2).

(iv) To test whether the inhibition of macromolecular synthesis produced by BPG could be a consequence of a respiratory blockage, oxygen consumption in the presence of BPG was measured. The results shown in Table 2 indicate slight decrease of oxygen uptake in the presence of 1.7 mg of BPG per ml. Therefore, this effect could not be the cause of the strong inhibition of BPG on the synthesis of macromolecules. However, when a concentration of 5.6 mg of BPG per ml was used, a strong inhibition on respiration was observed (Table 1).

(v) Another possible explanation of the inhibition caused by BPG on macromolecular synthesis could be a decrease in the ATP pool. However, the results observed in Table 2 show that there is an increase rather than a decrease of ATP in the presence of the inhibitor. Control experiments with DNP and CAP were performed. BPG shows an effect similar to that of CAP, suggesting that inhibition of macromolecular synthesis could be the cause of the increase of ATP concentration.

(vi) BPG action is temperature dependent. As seen in Table 3, there is a direct relationship between the temperature at which cells were incubated with BPG and its lethal effect. At 37 C, the percentage of survival is zero, whereas at 10 C it is 50%. In this experiment, 6 × 10^{6} cells/ml and 0.56 mg of BPG per ml were used.

Phage T4 inactivation. The effect of BPG on phage T4 was measured. Figure 5 shows an exponential inactivation. After 22 hr of treatment, phage T4 lost 99% of its infective capacity on *E. coli*. In an experiment not shown here Tris antagonized strongly with BPG, decreasing its inactivating action.

In vitro protein synthesis. To test whether BPG affected directly protein synthesis, experiments were performed with a cell-free system. Figure 6 shows that BPG inhibited [^{14}C]isoleucine incorporation. As with the in vivo experiments (Fig. 2), Tris also antagonized BPG action (Fig. 6). In one experiment, Tris was added 12 min after BPG addition. As can be seen (Fig. 6), Tris did not reverse BPG activity. This result is similar to that observed with whole cells and may indicate that at 12 min the effect of BPG is irreversible.

Effect of compounds related to Tris. In view of the antagonism caused by Tris on BPG action, other structurally related compounds were investigated. Table 4 shows the effect of BPG on the growth of a culture of *E. coli* in the presence of these compounds. Only 2-amino-2-methyl-1,3-propanediol antagonized with BPG as did Tris.

Effect of CAP on the production of BPG. To know whether BPG could be synthesized in the presence of a protein synthesis inhibitor, the effect of 50 μg of CAP per ml was tested. It was added simultaneously with glycerol to a culture of strain 43 grown on succinate. After 3 hr of incubation, the medium was filtered and tested for its lethal activity. It was observed that BPG is produced in the presence of CAP.

Comparison of BPG and MG. Since Freedberg et al. have characterized the bactericidal product as MG, we performed experiments to know whether our results with BPG could be due to its MG content.

The amounts of MG present in BPG preparation were determined with DNPH reaction (3). We found that 1 mg of BPG contained 1.76 μmoles of MG. In the following experiments, the MG content of BPG is expressed in parenthesis. The effects of BPG and MG on macromolecular synthesis were compared. Table 5 shows that MG also inhibits [^{14}C]isoleucine, [^{14}C]uracil, and [^{14}C]thymidine incorporation.

Previous studies with *E. coli* B/1,5 carried out by Együd and Szent-Györgyi (5) had shown that MG inhibited protein synthesis affecting RNA and DNA synthesis to a much lesser extent. The lack of correlation between the above results and those reported here could be due to differences in some conditions of the experiments or on the strain used. We ruled out the latter possibility by studying the effect of BPG and MG on *E. coli* B/1,5. As seen in Table 5, strain B/1,5 shows the same behavior as strain 3000 Y-14. Actions of BPG and MG were also compared in in vitro protein synthesis experiments. As indicated in Table 6, a similar effect was observed with both compounds.

The inhibition exerted by BPG on oxygen con-

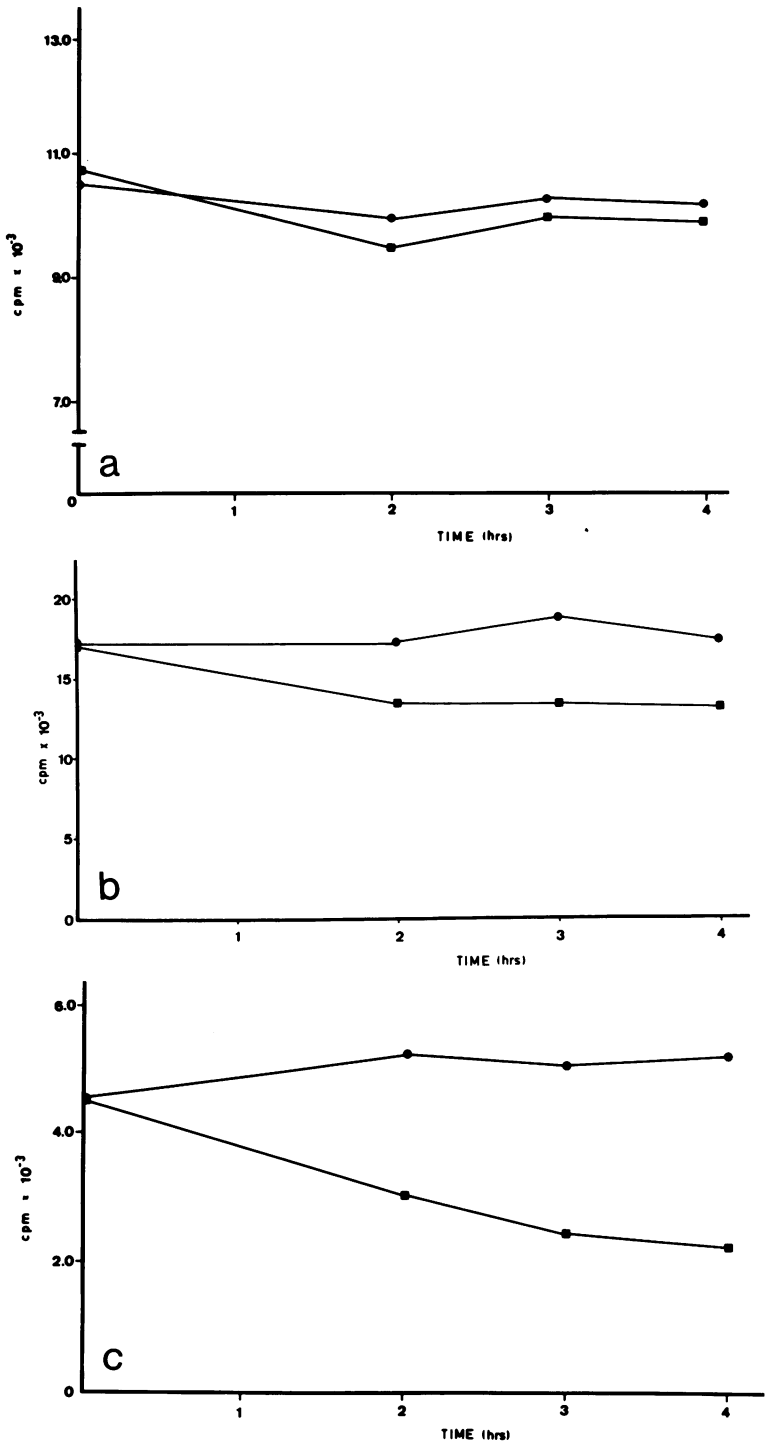

FIG. 4. *Effect of BPG on the degradation of macromolecules: Proteins (a); RNA (b), and DNA (c). Incubations were carried out with 2 × 10⁹ cells/ml. Control (●); 1.7 mg of BPG per ml (■).*

TABLE 1. *Effect of BPG on oxygen uptake*

Time of incubation (min)	O_2 (μliters/10^8 cells)		
	BPG (5.6 mg/ml)	BPG (1.7 mg/ml)	Without BPG
30	0.001	0.028	0.040
60	0.001	0.061	0.075

TABLE 2. *Effects of inhibitors on adenosine triphosphate pool in Escherichia coli*

Inhibitor[a]	ATP (moles × 10^{12}/10^8 cells) Time of incubation		
	0 Min	30 Min	60 Min
None	2.6	2.7	2.3
DNP (2 mM)	3.1	1.9	1.6
CAP (50 μg/ml)	3.3	4.1	4.7
BPG (1.7 mg/ml)	3.0	4.0	4.3

[a] DNP, 2,4-dinitrophenol; CAP, chloramphenicol.

TABLE 3. *Lethal effect of BPG at different temperatures*[a]

Temp (C)	Survival (%)
10	53
12	48
16	20
20	2.4
29	0.3
37	0.0

[a] Cells (6×10^8 per ml) were incubated for 3 hr with 0.56 mg of BPG per ml.

sumption (Table 1) agrees with a previous report (4) that indicates that 1 mM MG does not affect respiration but that a 10 times higher concentration does produce a strong inhibition.

The spectra of BPG and MG were compared. Figure 7 shows in the spectrum of BPG the presence of two substances, one of which has the absorption characteristics of MG. When BPG (*p*H 7.0) was heated at 100 C during 30 min, its lethal activity was lost (Fig. 8), though as mentioned previously (16) no effect was observed when BPG (*p*H 3.8) was heated during 75 min. MG at *p*H 7.0 was also inactivated when treated in the same conditions. Both BPG and MG were inactivated at *p*H 10.0. This result is in agreement with the previous report of Freedberg et al.

In all the experiments on the mode of action of BPG, heat-inactivated BPG controls were used. This inactivated BPG completely lost activity in all the cases, suggesting that no other active product was present in the BPG preparation.

The lethal effect produced by 1 mM MG was equivalent to that of 0.56 mg of BPG per ml (1 mM MG). Tris (30 mM, *p*H 7.0) antagonized that lethal activity when added simultaneously with BPG. However, Tris did not revert the lethal effect when added 3 hr after BPG addition.

The antagonism of Tris on the effects produced by BPG could be due to an interaction between both compounds. The reactions of Tris with BPG and MG were examined by measuring spectral changes. Figure 9 shows that the spectra of BPG and MG were markedly changed by treatment with Tris.

Interaction of MG with nucleotides. Previous results showed that glyoxal interacts with guanilic acid and guanine producing a shift in their spectra (12, 13). By using MG, similar results were obtained with guanosine triphosphate (GTP) (Fig. 10). No effect with ATP, cytidine triphosphate and uridine triphosphate was observed.

DISCUSSION

Our data indicate that in *E. coli* cells, DNA,

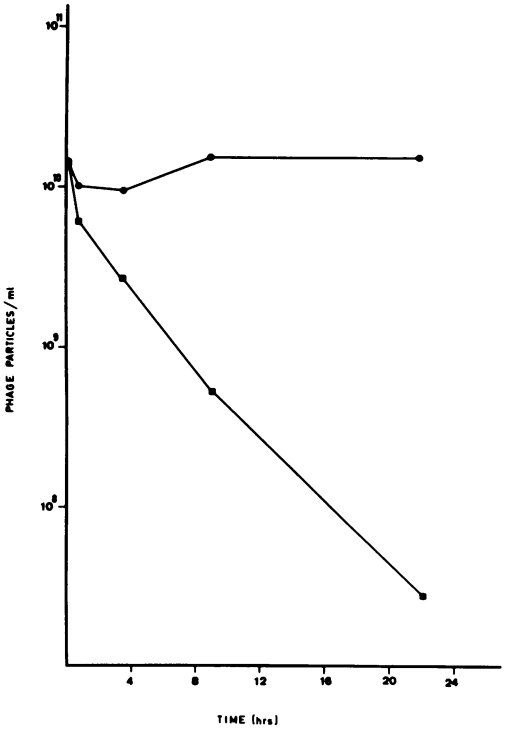

FIG. 5. *Effect of BPG on phage T4 inactivation. Control (●); 4.0 mg of BPG per ml (■). Phage T4 (1.5×10^{10} particles/ml) was incubated in LB medium (10) with 4.0 mg of BPG per ml at 37 C. Samples were taken at different times, diluted out, and plated on rich medium.*

RNA, and protein synthesis are strongly inhibited by addition of 1.7 mg of purified BPG per ml. The fact that at this concentration BPG modifies only slightly the ATP level, respiration, marcomolecular degradation, and substrate pool suggests that it acts directly on macromolecular synthesis; a clearer evidence comes from the observation that BPG inhibits in vitro protein synthesis.

According to Freedberg et al., MG is the active compound of the bactericidal product. After comparing BPG and MG, our results confirm this identification and establish that all the ac-

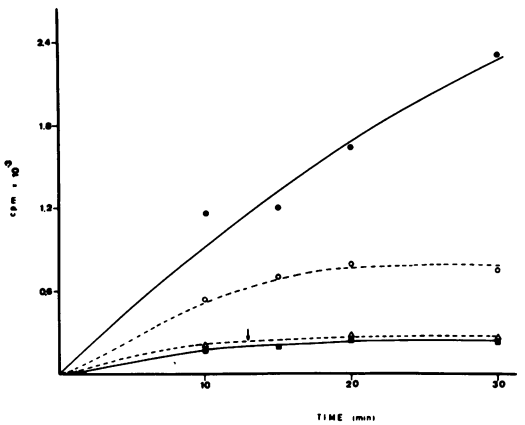

FIG. 6. *Effects of BPG on in vitro* L-[^{14}C]*isoleucine incorporation. Control* (●); *1.7 mg of BPG per ml* (■); *1.7 mg of BPG per ml* (○) *plus 0.1* M *Tris, pH 7.0, added at zero time; 1.7 mg of BPG per ml* (△) *plus 0.1* M *Tris, pH 7.0, added 12 min after.*

TABLE 4. *Effect of tris(hydroxymethyl)aminomethane (Tris) and structurally related compounds on BPG activity*

Addition[a]	Growth	
	With 0.56 mg of BPG per ml	Without BPG
None	−	+
2-Amino-2(hydroxymethyl)-1,3-propanediol (Tris)	+	+
2-Amino-2-methyl-1,3-propanediol	+	+
2-Amino-2-methyl-1-propanol	−	+
DL-2-Amino-1-propanol	−	+
2-Amino-ethanol	−	+

[a] The compounds were adjusted to *p*H 7.0 and added at a final concentration of 0.1 M.

TABLE 6. *Effects of BPG and methylglyoxal (MG) on in vitro protein synthesis*[a]

Inhibitor	Activity at[b]	
	15 min	30 min
None	2,294	5,395
BPG	441	328
MG	236	281

[a] BPG 1.7 mg/ml (3 mM MG) and 3 mM MG were used.

[b] Expressed as counts per minute per milligram of protein.

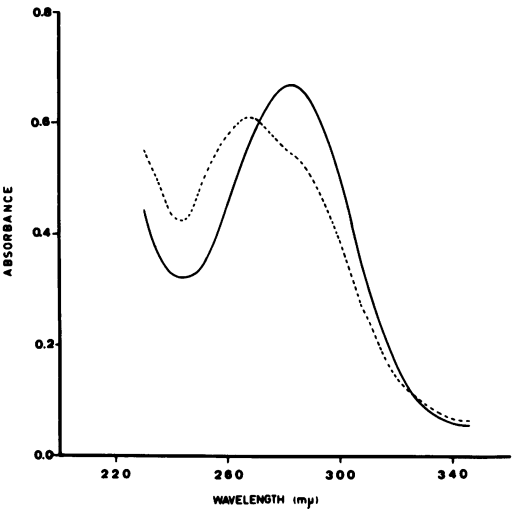

FIG. 7. *Methylglyoxal (MG) and BPG spectra. Solid line, 15 mM MG, pH 7.0; dashed line, 5.6 mg of BPG per ml (10 mM MG), pH 7.0. MG and BPG were purified by passage through a Dowex-1 (Cl^-) column.*

TABLE 5. *Effects of BPG and methylglyoxal (MG) on macromolecular synthesis in two strains of Escherichia coli*[a]

Additions	Strain 3000 *Y*-14 (counts/min)			Strain B/1,5 (counts/min)		
	[^{14}C]isoleucine	[^{14}C]uracil	[^{14}C]thymidine	[^{14}C]isoleucine	[^{14}C]uracil	[^{14}C]thymidine
None	32.816	22.318	138.121	29.146	11.128	105.083
BPG	2.170	5.213	36.539	1.503	3.511	13.041
MG	1.614	4.028	36.922	1.258	2.739	14.109

[a] Cells (2×10^8 per ml) were incubated at 37 C during 30 min; 0.56 mg of BPG per ml (1 mM MG) and 1 mM MG were used.

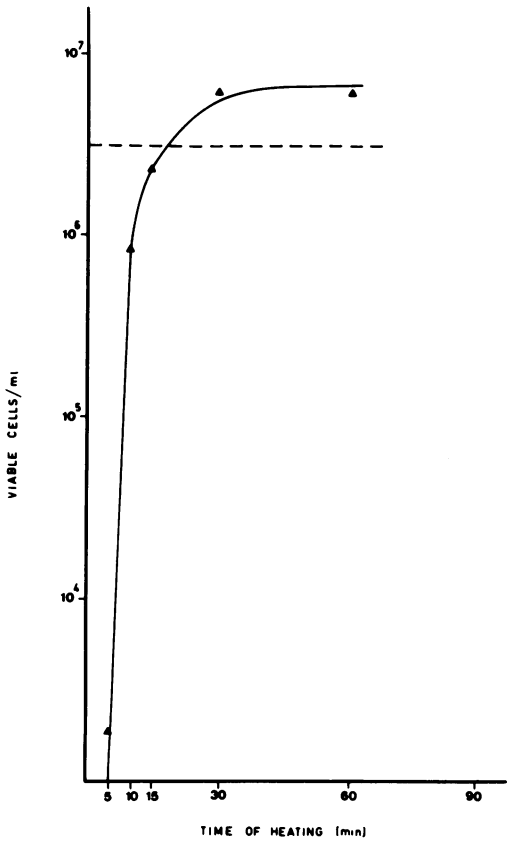

FIG. 8. *Heat inactivation of BPG. A 0.56-mg amount of BPG per ml (1 mM MG), pH 7.0 (▲), was heated at 100 C; at timed intervals, samples were removed and tested for lethal effect. Dashed line, no BPG added.*

tivity of our BPG preparation is due to its MG content. Therefore, hereafter we shall refer to BPG as MG.

A closely related compound, glyoxal, was studied by other workers, and their results show a behavior similar to MG. Thus, Klamerth (7)

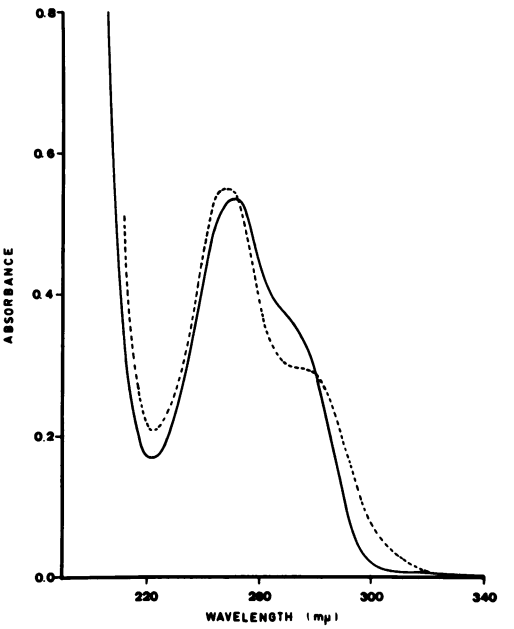

FIG. 10. *Spectral change of guanosine-5'-triphosphate (GTP) treated with MG in 0.1 M sodium phosphate, pH 7.4. Solid line, spectrum obtained with 25 μM GTP. Dashed line, spectrum obtained by treatment of 25μM GTP with 3 mM MG for 2 hr at 25 C. Absorption due to MG was subtracted.*

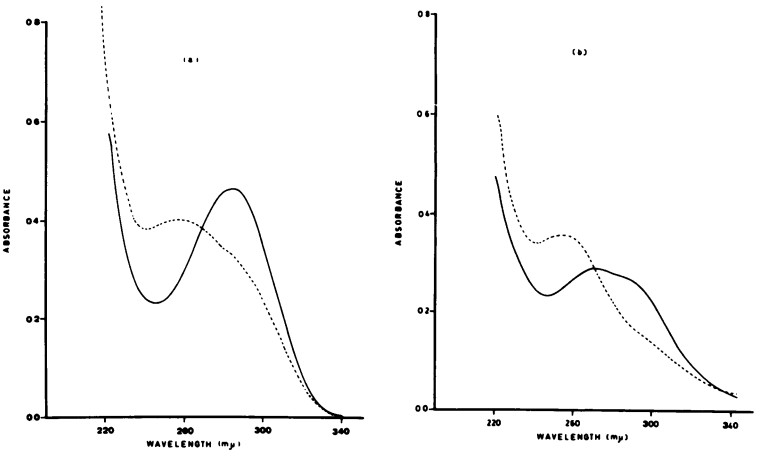

FIG. 9. *Spectral changes of MG and BPG treated with 0.1 M Tris at pH 7.0. (a) Solid line, 10 mM MG, pH 7.0; dashed line, 10 mM MG treated with 0.1 M Tris, pH 7.0, and immediately recorded. (b) Solid line, 2.8 mg of BPG per ml (5 mM MG), pH 7.0; dashed line, 2.8 mg of BPG per ml (5 mM MG), pH 7.0, treated with 0.1 M Tris, pH 7.0, and immediately recorded. MG and BPG were purified by passage through a Dowex-1 (Cl^-) column.*

found that glyoxal, like MG on *E. coli*, inhibits macromolecular synthesis on fibroblasts. It was also observed that glyoxal reacts with guanine (12) and guanosine monophosphate (12, 13); this effect is similar to the one of MG with GTP reported here. Besides, glyoxal produces a shift in the melting temperature of DNA (1) and inactivates naked RNA tobacco mosaic virus (13).

According to the above observations, the inhibition of DNA and RNA synthesis could be due to a reaction of MG with guanine residues contained in DNA and its precursors, whereas protein synthesis inhibition could be caused by a reaction of MG with guanine residues of RNA and its precursors.

The fact that MG inhibits protein synthesis almost instantly after its addition although DNA and RNA synthesis continue for a few more minutes could indicate a difference in the accessibility of MG to guanine residues of RNA and DNA.

It is surprising that MG can be produced in such large amounts by strain 43. Hopper and Cooper (6) found that 1 mM phosphate inhibits MG synthase, the enzyme that converts DHAP to MG, and that this inhibition can be overcome by high concentrations of DHAP. Strain 43, insensitive to fructose-1,6-diphosphate control, metabolizes glycerol at a higher rate than a normal strain (15), and as a consequence may accumulate DHAP permitting a rapid production of MG.

Some experiments on the production of MG by strain 43 were performed and they established that MG is produced even when glycerol is added in the presence of CAP. Since strain 43 is constitutive for the glycerol phosphate system (kinase and dehydrogenase), it is concluded that MG synthase is produced constitutively. This result agrees with previous observations (6).

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