Biochemical Pharmacology, Vol. 29, pp. 1791–1797.

0006-2952/80/0615-1791 $02.00/0

7-RIBOSYL-3-DEAZAGUANINE—MECHANISM OF ANTIBACTERIAL ACTION

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(*Received* 9 *November* 1979; *accepted* 26 *December* 1979)

Abstract—The mechanism by which 7-ribosyl-3-deazaguanine [7R3DG, 6-amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one] exerts its antibacterial effect was examined. *Escherichia coli* was found to contain an enzyme that exhibited the properties of a nucleoside phosphorylase and that converted 7R3DG to 3-deazaguanine [3DG, 6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one], but no mammalian system that was examined (Erlich ascites, rat liver and human liver) was able to convert 7R3DG to 3DG. The 3DG arising from the phosphorolysis of 7R3DG was converted to 3-deaza-GMP [3DGMP, 6-amino-1-β-D-ribofuranosylimidazo [4,5-*c*]pyridin-4(5*H*)-one-5′-phosphate] by the guanine phosphoribosyltransferase present in *E. coli*. A strain of *E. coli*, resistant to 7R3DG, was found to lack this enzyme and, therefore, was unable to convert 3DG to 3DGMP.

7-Ribosyl-3-deazaguanine (Fig. 1, 7R3DG¶) was shown by Matthews *et al.* [1] to be a highly active antibacterial agent that had significant activity against several gram-negative strains. However, it exhibits no antiviral activity *in vivo* against various DNA and RNA viruses [2] and no activity as an inhibitor of purine biosynthesis *de novo* [3]. In contrast, 3-deazaguanine (Fig. 1, 3DG) the free base of 7R3DG, exhibits both antibacterial [4] and antiviral [5, 6] activity and inhibits purine biosynthesis *de novo* [3]. A strain of *Escherichia coli* resistant to 3DG has been shown to be resistant to 7R3DG also [4]. When considered together, these results suggested to us that *E. coli* perhaps can convert 7R3DG to 3DG, which may be the active form of 7R3DG. Further, it appears that mammalians cells cannot convert 7R3DG to an active form.

Our studies on the mechanism of the antibacterial activity of 7R3DG in *E. coli* are described herein. We found that *E. coli* can convert 7R3DG to 3DG but that mammalian cells apparently do not contain the enzymes that mediate this conversion.

MATERIALS AND METHODS

Chemicals

The guanine and guanosine analogs shown in Fig. 1 were synthesized as described previously [2, 7]. 8-[^{3}H]-7R3DG (145 mCi/mmole) and 8-[^{3}H]-3DG

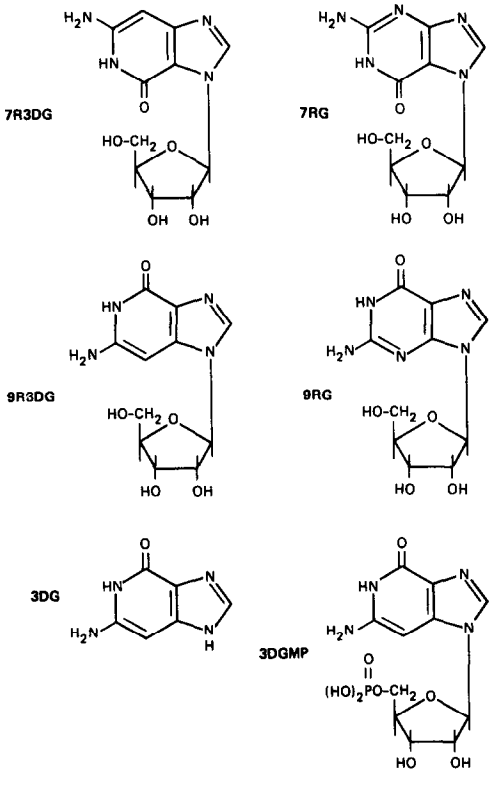

Fig. 1. Structures of compounds studied.

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¶ Abbreviations: 7R3DG = 6-amino-3-β-*D*-ribo-furanosylimidazo[4,5-*c*]pyridin-4(5*H*)-one; 3DG = 6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one; 9R3DG = 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one; BAP = bacterial alkaline phosphatase; GPRTase = guanine phosphoribosyltransferase; 3DGMP = 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one-5′-phosphate; 7RG = 7-β-D-ribofuranosylguanine; 9RG = guanosine; and PRPP = P5-phosphoribosyl-1-pyrophosphate.

(120 mCi/mmole) were prepared by ICN Radiochemicals, Irvine, CA. Toluscint II and Aquascint II, scintillation fluids, were also obtained from ICN Radiochemicals.

Isolation of 7R3DG-resistant strain of E. coli *Ec* 138

Resistance was induced by serial 24-hr passages of *E. coli* Ec 138 in the presence of 1 μM 7R3DG. Resistant organisms appeared within 48 hr. Resistance was defined as a 100-fold increase in the concentration of 7R3DG required to inhibit growth. The resistance was stable to serial passages in agar; this was proved by a subsequent check of the identity of the *E. coli* strain with the API 20E System (Analytab Products, Carle Place, NY), and of the retention of its decreased sensitivity to 7R3DG. The resistance was also stable to passage through animals and recovery from blood.

Measurement of the uptake of 7R3DG by 7R3DG-sensitive and 7R3DG-resistant strains of E. coli *Ec* 138

Standard medium (9 ml) [8] was inoculated with 0.5 ml of a stationary-phase culture of either 7R3DG-sensitive or -resistant *E. coli* Ec 138. After shaking at 37° for 3 hr, the culture was in the logarithmic phase of growth, at which time 1 ml of a 10 mg/ml solution of 8-[^{3}H]-7R3DG (0.13 μCi/ml) was added. At various times (0.5–60 min) after the addition of the [^{3}H]-7R3DG, 0.5-ml aliquots of the culture were removed and the cells were collected on a millipore filter. The cells were oxidized in a Packard sample oxidizer; the radioactivity in the resulting [^{3}H]H_2O was quantitated by liquid scintillation counting in Aquascint II. The values for the percentages of total radioactivity associated with the cells were normalized to 10 mg total dry weight of cells to compensate for the cell growth that occurred during the experiment. In a parallel experiment, growth of the bacteria was monitored as follows: 180 ml of standard medium were inoculated with 10 ml of a stationary-phase culture of either 7R3DG-sensitive or -resistant *E. coli* Ec 138. After shaking at 37° for 3 hr, the culture was in the logarithmic phase of growth, at which time 20 ml of a 10 mg/ml solution of 7R3DG were added. An identical culture of the 7R3DG-sensitive strain was left untreated. At various times (0–60 min) after the addition of 7R3DG, 10-ml aliquots of the culture were removed and the cells were collected on a preweighed millipore filter. The filters were dried overnight at 100° in a vacuum oven and reweighed to determine the dry weight of cells.

Separation of metabolites of 7R3DG in extracts of E. coli *Ec* 138

Standard medium (9 ml) [8] was inoculated with 0.5 ml of either 7R3DG-sensitive or 7R3DG-resistant strains of *E. coli* Ec 138, incubated, and exposed to 8-[^{3}H]-7R3DG as described above. After 2–20 min of incubation with the 7R3DG, the cells were collected and washed twice in 0.15 M NaCl by centrifugation at 10,000 *g* for 5 min. The cells were disrupted by glass–Teflon homogenization with 2 ml of 5% $HClO_4$. The supernatant (acid-soluble) fraction from centrifugation (10,000 *g* for 20 min) was neutralized with 2 N KOH; after sitting in ice for 30 min, the resulting precipitate was removed by centrifugation (10,000 *g* for 20 min). The neutralized supernatant fraction was concentrated by lyophilization and reconstituted in 5% of the original volume. Thin-layer chromatography plates (Woelm silica gel, 0.10 mm thick, 20 × 20 cm) were prepared for use by developing them in CH_3CN–NH_4OH–2-propanol–H_2O (7 : 1 : 1 : 1, by vol.) and then drying them in a stream of cool air. The concentrated extract was applied to these plates and developed in the solvent system described above. The R_f values of the various compounds were: 3-deazaxanthine, 0.54; 3DG, 0.49; 7R3DG, 0.38; 9R3DG, 0.17; 7R3DG-5′-phosphate and 9R3DG-5′-phosphate, 0.0 (see Fig. 1 for structures). The absorbent from the regions of the plates corresponding to each of the metabolites was scraped off and eluted with 3 ml of H_2O. The radioactivity was quantitated by liquid scintillation counting in 15 ml of Aquascint II. In some cases, the nucleotides in the extracts from the [^{3}H]-7R3DG-treated *E. coli* were converted to their corresponding nucleosides by the addition of 1 μg (in 1 μl) of *E. coli* BAP (Worthington BAPC) to 25 μl of extract and incubation at 30° for 1 hr. The BAP-treated extract was then chromatographed as described above.

Measurement of the uptake of [^{14}C]*guanine and* [^{14}C]*hypoxanthine by 7R3DG-sensitive and 7R3DG-resistant strains of* E. coli *Ec* 138

Cultures of *E. coli* Ec 138, 7R3DG-sensitive and 7R3DG-resistant strains, were grown to stationary phase in standard media [8]. Aliquots (2.5 ml) were transferred to culture tubes containing 2×10^7 c.p.m. of either [^{14}C]hypoxanthine or [^{14}C]guanine, both 55 mCi/mmole. Duplicate 0.2-ml aliquots were removed at 15-min intervals from 0 to 60 min and applied to millipore filter discs. The discs were immediately washed with 10 ml of 10 mM phosphate buffer (pH 7.1) containing 0.15 M NaCl. After being air-dried, the discs were cut up and placed in scintillation vials. The radioactivity was quantitated by liquid scintillation counting in 5 ml of Toluscint II.

Preparation and assay of guanine PRPP transferase from E. coli *Ec* 138

Guanine PRPP transferase was purified from *E. coli* Ec 138 cells by the method of Miller *et al.* [9] except that cells were broken by the use of a Branson ultrasonic probe. Unbroken cells and cell debris were removed by centrifugation at 50,000 *g* (10 min) and the supernatant fraction was treated with streptomycin sulfate. Cellex E chromatography of the streptomycin sulfate supernatant fraction was then performed. The ratios of guanine/hypoxanthine PRPP transferase activities, determined before and after Cellex E chromatography, were 3.2 and 20, respectively. The latter fraction was stored at −20°. The assay [10, 11] mixture contained, in a total volume of 0.2 ml: 50 mM Tris–HCl (pH 7.2), 5 mM PRPP, 0.1 mM 8-[^{3}H]guanine (30 mCi/mmole) or 8-[^{3}H]-3DG (120 mCi/mmole). After an appropriate incubation time (5–20 min, previously determined from pilot assays to assure kinetically valid results) at 37°, 25-μl aliquots were absorbed onto DEAE (Whatmann DE-81) discs. The discs were air-dried and

washed successively in 2 mM ammonium formate (pH 9.2), ethanol–water (1 : 1), and ethanol. The radioactivity in the dried discs was quantitated by liquid scintillation counting in 3 ml of Toluscint II. Controls lacked PRPP. One unit was defined as that amount of enzyme activity that produced 1 nmole of product in 1 min under the conditions described above.

Preparation and assay of 7R3DG cleavage activity

Extracts for the assay of 7R3DG cleavage activity were prepared from various sources as described below.

E. coli. Packed cells of *E. coli* Ec 138 (5–10 g, wet wt) were ground with twice the weight of alumina (Sigma Chemical Co., St. Louis, MO). The alumina cell paste was extracted with 50 ml of 20 mM potassium phosphate buffer (pH 7.4) and stirred for 30 min. The extract was centrifuged at 8000 *g* for 15 min and the supernatant fraction was recentrifuged at 30,000 *g* for 60 min. The supernatant was fractionated with ammonium sulfate; the fraction precipitating between 55 and 90% saturation was used in assaying the 7R3DG cleavage activity after dissolving the pellet in 50 mM potassium phosphate buffer (pH 7.4) and storing it at −20°.

Rat liver. Fresh liver (5–10 g) was minced in 3 ml/g of 10 mM phosphate buffer (pH 7.4) and homogenized in a Kontes glass–Teflon homogenizer. The homogenate was centrifuged at 37,000 *g* for 30 min and the supernatant fraction was recentrifuged at 37,000 *g* for 90 min. The resulting supernatant was fractionated with ammonium sulfate. The 55–90% ammonium sulfate fraction was dissolved in 50 mM phosphate buffer (pH 7.4) and stored at −20°.

Human liver. An extract was prepared from a frozen biopsy sample, in the same manner as for rat liver.

Erlich ascites cells. A 25,000 *g* supernatant fraction, prepared as described previously [3], was used as the enzyme source.

The 7R3DG cleavage activity was assayed as previously described [3]: 200–300 μg of protein from each of the above sources were incubated with 250 nmoles of nucleoside substrate in 6 ml of 10 mM potassium phosphate buffer (pH 7.4) at 37° for 18–20 hr. Reaction mixtures, which had been heated to 60° for 4 min before overnight incubation, were used as controls. Product formation was determined by noting the u.v. spectral shifts [3] associated with the conversion of each nucleoside to its corresponding free base.

RESULTS AND DISCUSSION

Effects of purines and pyrimidines on the antimicrobial activity of 7R3DG

Our antimicrobial testing was routinely performed in a completely chemically defined medium free of any added purines and/or pyrimidines [8]. We found that the antimicrobial potency of 7R3DG was greatly

Table 1. Effects of nucleic acid components and their precursors on the antibacterial activity *in vitro* of 7R3DG*

	Minimum concentration required to completely prevent antibacterial activity of 20 μM 7R3DG		
Compound	Conc. (μM)	Compound	Conc. (μM)
Adenine	>200	Uracil	>200
Adenosine	40	Uridine	20
Deoxyadenosine	20	Deoxyuridine	40
Adenylic acid	>200	Uridylic acid	>200
		Deoxyuridylic acid	>200
Guanine	40		
Guanosine	4	Cytosine	>200
Deoxyguanosine	8	Cytidine	40
Guanylic acid	>200	Deoxycytidine	80
		Cytidylic acid	>200
Hypoxanthine	200	Deoxycytidylic acid	>200
Inosinic acid	>200		
		Thymidine	40
Xanthine	20		
Xanthylic acid	>200	Orotic acid	>200
		Orotidine	>200
5-Aminoimidazole-4-carboxamide	>200		
1-β-D-Ribofuranosyl-5-aminoimidazole 4-carboxamide	>200		

* Standard medium (9 ml) [8] was inoculated with 0.5 ml of a stationary phase culture of 7R3DG-sensitive *E. coli* Ec 138 and shaken at 37° for 3 hr until the culture was in the logarithmic phase of growth, at which time 1 ml of a solution containing 200 μM 7R3DG and various concentrations (2 μM–2 mM in medium) of the test agent were added. The culture was incubated for 1 hr at 37° with shaking. The growth (A_{540}) was compared to that of a culture receiving 1 ml of medium only.

Table 2. Metabolism of 7R3DG in *E. coli* Ec 138*

Metabolite	Percent of total radioactivity			
	Sensitive cells		Resistant cells	
	−BAP	+BAP	−BAP	+BAP
3DG	13	10	72	68
7R3DG	5	3	7	9
9R3DG	10	67	6	14
"Nucleotides"	65	15	11	3
Other (unidentified)	7	5	4	6

* Cells were grown and exposed to [^{3}H]-7R3DG, and extracts were prepared from the cells and subjected to chromatographic analysis, as described in Materials and Methods. The metabolites appearing in extracts treated with bacterial alkaline phosphatase (BAP) prior to chromatography are designated "+BAP", while metabolites appearing in untreated extracts are designated "−BAP".

diminished when tested in complex media. This observation suggested to us that some component of the complex media was interfering with the antimicrobial activity of 7R3DG. Because it seemed likely that the interfering factor was a nucleic acid component, we tested the ability of various nucleic acid components to reverse the antimicrobial activity of 7R3DG in a chemically defined medium. Each compound was tested at various concentrations up to 200 μM to determine the concentration that completely prevented the antibacterial activity of 20 μM 7R3DG. This concentration of 7R3DG was ten times its minimum inhibitory concentration against *E. coli* Ec 138 [1], and was chosen because it allowed us to readily titrate the potency of the various compounds as inhibitors of the antibacterial action of 7R3DG.

The results of these studies are summarized in Table 1. Guanosine and deoxyguanosine were the most potent reversal agents, completely preventing the antibacterial activity at concentrations 20 and 40%, respectively, of that of 7R3DG. These data suggest that 7R3DG may be interfering with guanine nucleotide synthesis, metabolism, and/or utilization for RNA or DNA synthesis. 3DG has been shown to inhibit DNA synthesis in *E. coli*, with little effect on RNA synthesis [4], and to inhibit DNA synthesis—but not RNA synthesis—in L1210 cells [12].

It will be shown below that 7R3DG was converted to 3DG by a nucleoside phosphorylase and that 3DG was converted to 3DGMP by GPRTase. If the phosphorolysis of 7R3DG was mediated by a guanosine/deoxyguanosine phosphorylase, then the reversal activity of guanosine and deoxyguanosine may reflect competition for the phosphorylase.

Other purines—adenosine, deoxyadenosine, guanine and xanthine—also interfered with the antimicrobial activity of 7R3DG, but these agents were somewhat less potent than guanosine and deoxyguanosine, completely reversing the antibacterial activity at concentrations one to two times that of 7R3DG. Since xanthine has been reported to be an inhibitor of GPRTase with a K_i of 48 μM [9], xanthine may have inhibited the conversion of 3DG to 3DGMP. Guanine may be less potent than the guanine nucleosides because it may be taken up by the bacteria less efficiently than the nucleosides. The mechanism by which adenosine, deoxyadenosine and xanthine reverse the action of 7R3DG is unknown, but they may eventually be converted to guanine nucleotides through the purine salvage pathways.

Interestingly, some pyrimidine nucleosides—uridine, deoxyuridine, cytidine, deoxycytidine, and thymidine—were active reversal agents at concentrations one to four times that of 7R3DG. The mechanism by which these pyrimidine nucleosides exhibited their reversal action is also not understood, but they may have affected either the uptake or metabolism of 7R3DG.

Uptake and metabolism of 7R3DG by sensitive and resistant strains of E. coli

When logarithmically growing cultures of 7R3DG-sensitive and 7R3DG-resistant *E. coli* Ec 138 were exposed to 1 mg/ml (4.3 mM) 8-[^{3}H]-7R3DG, the profiles of total radioactivity associated with the two types of cells were quite different (Fig. 2). After

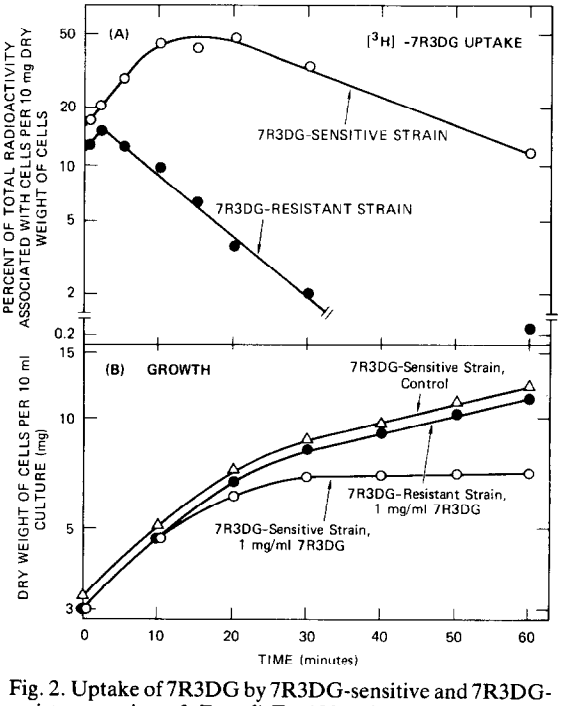

Fig. 2. Uptake of 7R3DG by 7R3DG-sensitive and 7R3DG-resistant strains of *E. coli* Ec 138. The experiment was performed as described in Materials and Methods.

introduction of the drug into the medium, the sensitive cells took it up at a rate that increased logarithmically with the time until, at 10 min, approximately 50 per cent of the radioactivity introduced into the medium had become associated with the cells. In Fig. 2, the values for the percentage of total radioactivity associated with the cells were normalized to account for the increase in the volume of cells during the experiment. By 20 min, the percentage of the radioactivity associated with the sensitive cells began to decrease; by 60 min, the level was about one-fourth that observed at 10–20 min. In contrast, the resistant cells took up the radioactivity only for the first 2 min after addition of the drug to the medium, after which time the cell-associated radioactivity decreased logarithmically until at 1 hr less than 1 per cent of the total radioactivity was present in the cells.

Table 2 shows the metabolites of 7R3DG found in extracts of sensitive and resistant cells harvested 10 min after introduction of the drug into logarithmically growing cultures. Because the 5′-nucleotides of 7R3DG and of 3-deazaguanosine (Fig. 1, 9R3DG) are difficult to separate chromatographically, the identity of the nucleotides was ascertained by enzymically converting the nucleotides in the extracts to their corresponding nucleosides (+BAP) prior to chromatography and looking for an increase in the amount of total radioactivity appearing as the various nucleosides relative to the untreated (−BAP) extract.

In the sensitive cells, 65 per cent of the radioactivity was in the form of nucleotides; alkaline phosphatase treatment (+BAP) of the extract prior to chromatography resulted in an increase in the relative amount of 9R3DG but in no significant change in the amount of 7R3DG. The sensitive cells contained very little 7R3DG and moderate amounts of 3DG, 9R3DG, and an unidentified metabolite with an R_f of 0.56–0.57. This latter material may have been 3-deazaxanthine, which has an R_f of 0.54.

The resistant cells contained about the same relative amounts of 7R3DG and 9R3DG, but considerably less nucleotide material, which—as in the sensitive cells—was apparently phosphates of 9R3DG. The decrease in the relative amount of 9R3DG nucleotides in the resistant cells compared to the sensitive cells was approximately equal to the relative increase in 3DG in the resistant cells. The metabolite composition was also examined in cells harvested 2 min and 20 min after introduction of 7R3DG (data not shown), and the metabolite composition of both the sensitive and resistant cells was essentially the same as that observed at 10 min.

The results show that the resistant *E. coli* were able to efficiently convert 7R3DG to 3DG, which accumulated in the cells. The sensitive *E. coli* were also able to convert 7R3DG to 3DG, but instead of accumulating 3DG, these cells converted the 3DG to nucleotides of 9R3DG. The resistant cells were unable to convert 3DG to its nucleotide forms.

Uptake of hypoxanthine and guanine by 7R3DG-sensitive and 7R3DG-resistant strains of E. coli *Ec* 138

Because GPRTase is responsible for the conversion of guanine to GMP in *E. coli* [13], this enzyme was chosen as the most likely candidate for catalyzing the conversion of 3DG to 3-deazaguanosine-5′-phosphate (Fig. 1, 3DGMP), the capability that the 7R3DG-resistant *E. coli* lacked. To test this putative mechanism, we examined the abilities of the sensitive and resistant strains of *E. coli* Ec 138 to take up guanine. Since *E. coli* is known to contain separate purine phosphoribosyltransferases for guanine and for hypoxanthine [13], we also examined the ability of the sensitive and resistant *E. coli* to take up hypoxanthine, anticipating that this activity might not be affected in the 7R3DG-resistant strain.

The results (Fig. 3) indicate that the sensitive strain took up both guanine and hypoxanthine at approximately the same rate. In contrast, although the resistant strain was able to take up hypoxanthine as efficiently as the sensitive strain (Fig. 3, panel B), the ability of the resistant strain to take up guanine was drastically reduced (Fig. 3, panel A). It therefore

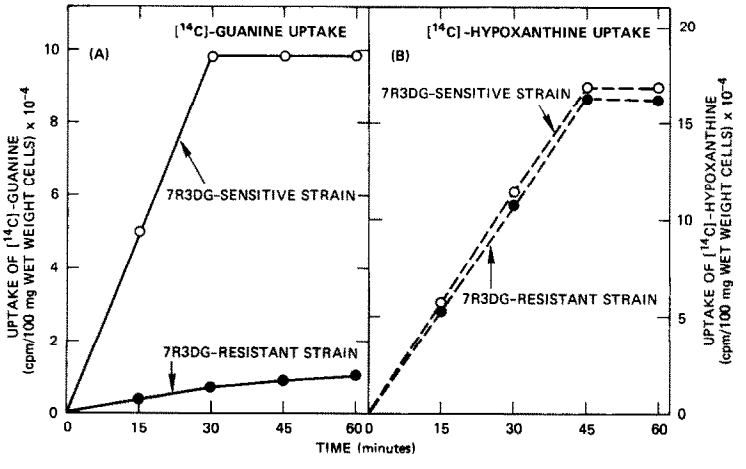

Fig. 3. Uptake of [^{14}C]guanine (A) and [^{14}C]hypoxanthine (B) by 7R3DG-sensitive and 7R3DG-resistant strains of *E. coli* Ec 138. The experiment was performed as described in Materials and Methods.

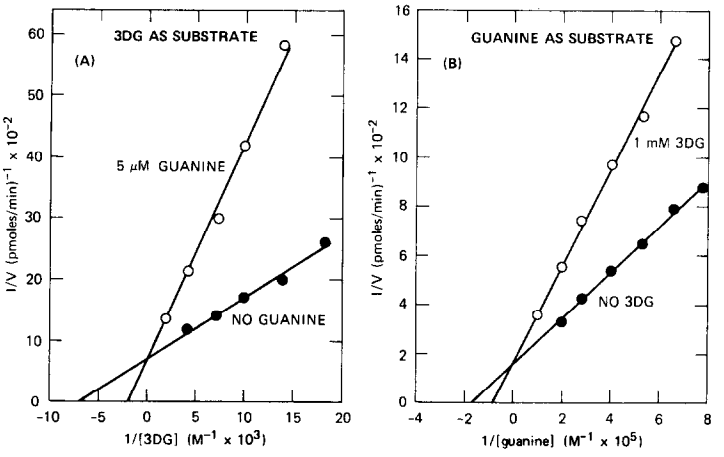

Fig. 4. Kinetics of 3DG (A) and guanine (B) as substrates and inhibitors of GPRTase. The assays were performed as described in Materials and Methods.

appears that the basis for the resistance to 7R3DG in the one strain is that it lacks GPRTase and has the accompanying inability to convert the 3DG, derived from 7R3DG, to 3DGMP. These results are consistent with the metabolism of 7R3DG presented above. In addition, the results complement those of Saunders *et al.* [4], who have shown that a strain of *E. coli* resistant to 3DG has lost the ability, present in the sensitive parental strain, to take up guanine but retains the ability of the parental strain to take up hypoxanthine.

3DG as substrate for GPRTase from E. coli *Ec* 138

3DG was examined for its ability to serve as a substrate for a partially purified GPRTase from *E. coli* Ec 138. As shown in Fig. 4 and Table 3, 3DG was a substrate for the enzyme, exhibiting a K_m of 140 μM, which is more than an order of magnitude larger than the K_m of 5.8 μM for guanine. In addition, the V_{max} for 3DG (6.3 units/mg) was approximately one-fifth that for guanine (28 units/mg). The data in Fig. 4 also show that 3DG was a competitive inhibitor of the phosphoribosylation of guanine (Fig. 4, panel B) and that guanine was a competitive inhibitor of the phosphoribosylation of 3DG (Fig. 4, Panel A). The K_i for guanine as an inhibitor of 3DG phosphoribosylation (5 μM) was the same as the K_m for guanine as a substrate for GPRTase. In contrast, the K_i for 3DG as an inhibitor of guanine phosphoribosylation (1000 μM) was approximately seven times greater than the K_m for 3DG as a substrate for the enzyme. This difference, considered by itself, is not entirely consistent with the proposed mechanism for the conversion of 3DG to 3DGMP by GPRTase. However, when these results are considered together with the observed metabolism of 7R3DG shown in Table 2, the lack of agreement between the K_i and K_m of 3DG for GPRTase does not force us to reject our proposed mechanism of action of 7R3DG.

Table 3. Kinetic parameters for 3DG and guanine with *E. coli* Ec 138 GPRTase*

Substrate	Inhibitor	K_m (μM)	V_{max} (units/mg)	K_i (μM)
Guanine		5.8	28	
Guanine	3DG			1000
3DG		140	6.3	
3DG	Guanine			5

* Assays were performed as described in Materials and Methods.

Lack of 7R3DG cleavage activity in mammalian systems

Although 7R3DG has potent antibacterial activity [1], it is innocuous in all mammalian systems so far examined [2, 3]. In contrast, 3DG is active in both bacterial and mammalian systems [3–6]. The growth-inhibiting activity of 3DG in mammalian systems is apparently a result of its conversion to 3DG-containing nucleotides [14]. Because we have shown here that *E. coli* can also convert 3DG to 3DG-

Table 4. Cleavage of guanosine analogs by *E. coli* and mammalian extracts*

Compound	Enzyme source *E. coli*	Erlich ascites	Rat liver	Human liver
7R3DG	+	–	–	–
7RG	+	–	–	–
9R3DG	–	+	+	+
9RG (guanosine)	+	+	+	+

* Extracts were prepared and the potential cleavage activity was assayed as described in Materials and Methods. The + indicates the ability of the extract to convert the substrate nucleoside to its respective base (7R3DG and 9R3DG to 3DG; 7RG and 9RG to guanine) as evidenced by the u.v. spectral shifts associated with the conversion of each nucleoside to its corresponding free base [3]. The – indicates the inability of the extract under the incubation conditions used, to convert the substrate nucleoside to its respective base, as evidenced by the lack of any u.v. spectral change.

containing nucleotides and because 3DG has been shown to inhibit DNA synthesis in *E. coli* [4], we postulated that the inactivity of 7R3DG in mammalian systems is due to the inability of mammalian cells to mediate the conversion of 7R3DG to 3DG, which we found to occur in *E. coli* as reported above. To test this hypothesis, we examined some mammalian systems for their ability to convert 7R3DG to 3DG. For comparison purposes, we also examined 7-ribosylguanine (Fig. 1, 7RG), 9R3DG and 9-ribosylguanine (Fig. 1, 9RG). As shown in Table 4, none of the three mammalian enzyme sources examined was able to produce any measurable conversion of 7R3DG to 3DG or of 7RG to guanine, but all three of these sources were able to convert 9R3DG to 3DG and 9RG to guanine. In contrast, the *E. coli* extract was able to cleave both 7-ribosyl nucleosides (7R3DG and 7RG) to their respective bases (3DG and guanine) and was also able to convert guanosine to guanine. However, the *E. coli* extract did not convert 9R3DG to 3DG. These results show that *E. coli* contains an enzyme activity that converts 7R3DG to 3DG and this activity is lacking in the mammalian systems examined. These results are consistent with the reported lack of activity of 7R3DG in mammalian systems [2, 3].

Properties of 7R3DG cleavage activity from E. coli *Ec* 138

The identity of the enzyme in *E. coli* that converts 7R3DG to 3DG was investigated further. There are three types of enzyme reactions that could mediate this conversion. One possibility is hydrolysis catalyzed by *N*-ribosylpurine ribohydrolase (EC 2.3.2.1, nucleoside hydrolase, nucleosidase). Second, the conversion could be a result of phosphorolysis mediated by a nucleoside phosphorylase such as purine nucleoside:orthophosphate ribosyltransferase (EC 2.4.2.1, purine nucleoside phosphorylase). A third possibility is the transfer of the ribosyl moiety from 7R3DG to a purine or pyrimidine base catalyzed by nucleoside:purine (pyrimidine) ribosyltransferase (EC 2.4.2.5, nucleoside ribosyl transferase). To differentiate among these possibilities, we examined the effects of phosphate, purine bases and pyrimidine bases on the ability of the *E. coli* Ec 138 extract to convert 7R3DG to 3DG. The *E. coli* extract was exhaustively dialyzed against a buffer containing 20 mM Tris–HCl (pH 7.4) and 10 mM $MgCl_2$ until it was phosphate-free, as assayed by the method of Lowry and Lopez [15]. When this phosphate-free extract was assayed, as described in Materials and Methods (with 10 mM Tris–HCl, pH 7.4, replacing the 10 mM potassium phosphate, pH 7.4), for its ability to convert 7R3DG to 3DG, it was completely inactive. This suggested that the 7R3DG cleavage activity is not a nucleoside hydrolase. Next, the addition of the following purine and pyrimidine bases at 0.1 or 1.0 mM to the phosphate-free assay did not result in any conversion of 7R3DG to 3DG: adenine, guanine, hypoxanthine, xanthine, uracil, cytosine, thymine or orotic acid. These results indicate that the 7R3DG cleavage activity is not a nucleoside ribosyltransferase. When 10 mM phosphate was added to the phosphate-free assay, the *E. coli* extract was able to cleave 7R3DG to 3DG, suggesting that the cleavage is occurring by a phosphorolytic mechanism. An examination of the ability of the extract to convert the naturally occurring nucleosides to their respective bases showed that the extract contained phosphorolytic (phosphate-dependent cleavage) activity for adenosine, guanosine, inosine, uridine, cytidine and thymidine (D. G. Streeter, M. Miller and J. P. Miller, unpublished results). These results do not allow us to determine which of the purine or pyrimidine nucleoside phosphorylases is responsible for the phosphorolytic cleavage of 7R3DG. This question is currently under investigation.

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