Role of Guanosine Kinase in the Utilization of Guanosine for Nucleotide Synthesis in *Escherichia coli*

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Using purine auxotrophic strains of *Escherichia coli* with additional genetic lesions in the pathways of interconversion and salvage of purine compounds, we demonstrated the *in vivo* function of guanosine kinase and inosine kinase. Mutants with increased ability to utilize guanosine were isolated by plating cells on medium with guanosine as the sole purine source. These mutants had altered guanosine kinase activity and the mutations were mapped in the gene encoding guanosine kinase, *gsk*. Some of the mutants had acquired an additional genetic lesion in the purine *de novo* biosynthetic pathway, namely a *purF*, a *purL* or a *purM* mutation. A revised map location of the *gsk* gene is presented and the gene order established as *proC*–*acrA*–*apt*–*adk*–*gsk*–*purE*.

INTRODUCTION

A number of purine sources can supply the requirement of purine auxotrophic strains of *Escherichia coli*. Among these compounds are purine ribonucleosides and deoxyribonucleosides. Adenosine, inosine and guanosine and the corresponding 2'-deoxy compounds can serve as the sole purine source (Nygaard, 1983). The utilization of the purine deoxyribonucleosides is dependent upon the functioning of purine nucleoside phosphorylase, encoded by the *deoD* gene. Strains that are *deoD* cannot incorporate exogenous purine deoxyribonucleosides into DNA (Karlström, 1970), nor will *pur deoD* strains utilize purine deoxyribonucleosides for growth (Hoffmeyer & Neuhard, 1971).

Several enzymes are responsible for the conversion of purine ribonucleosides to the corresponding ribonucleoside 5'-monophosphates. Inosine, adenosine and guanosine can be phosphorylated to the corresponding bases and ribose 1-phosphate by purine nucleoside phosphorylase. The bases in turn may be phosphoribosylated to the nucleoside 5'-monophosphates by appropriate phosphoribosyltransferases. Also guanosine and inosine kinase activities have been identified and the *gsk* gene, encoding guanosine kinase, mapped in *E. coli* (Jochimsen *et al.*, 1975). Nucleotide synthesis from adenosine proceeds via phosphorylation as above or through initial deamination to inosine, catalysed by adenosine deaminase (Hoffmeyer & Neuhard, 1971; Nygaard, 1983; Livshits, 1976). A different pathway has been proposed for converting adenosine to AMP, involving cleavage of adenosine to adenine by an enzyme different from purine nucleoside phosphorylase (Livshits & Sukhodolets, 1973a). The physiological implications of this enzyme have not been solved, but the enzyme responsible is very likely adenosylhomocysteine nucleosidase. This enzyme has affinity towards adenosine *in vitro* (Jochimsen, 1979). Relevant auxiliary pathways as well as the *de novo* purine pathway of *E. coli* are given in Fig. 1. Detailed reviews of the metabolism of purine nucleotides have recently appeared (Nygaard, 1983; Neuhard & Nygaard, 1987).

Abbreviation: AIR, aminoimidazole ribonucleoside 5'-monophosphate.

Fig. 1. Auxiliary and *de novo* pathways of purine nucleotides in *E. coli*. The abbreviations used for the *de novo* intermediates: R5P, ribose 5-phosphate; PRPP, phosphoribosylpyrophosphate; PRA, phosphoribosylamine; GAR, glycylamide ribonucleoside 5'-monophosphate; FGAR, formylglycinamide ribonucleoside 5'-monophosphate; FGAM, formylglycinamidine ribonucleoside 5'-monophosphate; AIR, aminoimidazole ribonucleoside 5'-monophosphate; CAIR, carboxaminoimidazole ribonucleoside 5'-monophosphate; SAICAR, succinylaminoimidazolecarboxamide ribonucleoside 5'-monophosphate; AICAR, aminoimidazolecarboxamide ribonucleoside 5'-monophosphate; FAICAR, formylaminoimidazolecarboxamide ribonucleoside 5'-monophosphate; IMP, inosine 5'-monophosphate; XMP, xanthosine 5'-monophosphate; SAMP, succinyl-AMP. The *de novo* pathway has been extended to include PRPP synthetase, encoded by the *prs* gene (Hove-Jensen, 1985; Hove-Jensen *et al.*, 1986). The individual *de novo* reactions are indicated by the genes encoding the respective enzymes: *purF*, glutamine PRPP amidotransferase; *purD*, GAR synthase; *purN*, GAR formyltransferase; *purL*, FGAR amidotransferase; *purM*, AIR synthase; *purEK*, AIR carboxylase; *purC*, SAICAR synthase; *purB*, adenylsuccinate lyase; *purH*, AICAR formyltransferase; *purA*, adenylsuccinate synthase; *guaB*, IMP dehydrogenase; *guaA*, GMP synthetase. In *E. coli* no gene has been assigned to the IMP cyclohydrolase. The broken arrow indicates the reactions leading from AIR to thiamin pyrophosphate (TPP, Newell & Tucker, 1968). The auxiliary reactions are indicated by numbers: 1, guanosine kinase (encoded by *gsk*); 2, purine nucleoside phosphorylase (*deoD*); 3, GMP reductase (*guaC*); 4, adenine phosphoribosyltransferase (*apt*); 5, hypoxanthine phosphoribosyltransferase (*hprt*); 6, guanine-xanthine phosphoribosyltransferase (*gpt*); 7, xanthosine phosphorylase (*xapA*); 8, adenosine deaminase (*add*). The reactions leading from ATP to AICAR are part of the histidine biosynthetic pathway. The functioning of this pathway is inhibited by the presence of histidine. The *xapA* gene is not expressed unless xanthosine is given as carbon source. Only under these conditions will xanthosine serve as purine source.

Two potent nucleoside transport systems, encoded by the *nupC* and *nupG* genes, have been determined genetically in *E. coli* (Munch-Petersen & Mygind, 1976; Hansen *et al.*, 1987). The two systems differ slightly in their specificities. Thus, the *nupG*-encoded system is responsible for uptake of all ribonucleosides and deoxyribonucleosides, whereas the *nupC*-encoded system takes up all ribonucleosides and deoxyribonucleosides except guanosine and deoxyguanosine (Munch-Petersen & Mygind, 1983).

Purine ribonucleoside kinases have been only sparsely described in *E. coli* (Jochimsen *et al.*, 1975), *Salmonella typhimurium* (Hoffmeyer & Neuhaard, 1971; Gots *et al.*, 1977) or other organisms (Fink & Nygaard, 1978). In this study, we have examined the physiological role of guanosine kinase (ATP:guanosine 5'-phosphotransferase, EC 2.7.1.-), by analysing the functioning of the enzyme *in vivo*. We further present a characterization of mutants with improved utilization of guanosine as a purine source. Among these mutants, we found some that had increased activity of guanosine kinase. Guanosine-utilizing mutants have been previously described. Two types of mutants were isolated in *E. coli* by Livshits & Sukhodolets (1973*b*): one type was found among rifampin-resistant mutants; the other was isolated by using guanosine as the sole purine source, and the genetic lesion mapped close to the *purE* gene. Both in *E. coli* and in *S. typhimurium* guanosine-utilizing mutants with defective phosphoribosylpyrophosphate synthetase have been reported (Hove-Jensen & Nygaard, 1982; Jochimsen *et al.*, 1985).

METHODS

Bacterial strains. All the *E. coli* K12 strains used and their genotypes are listed in Table 1.

Media, cell growth and the preparation of cell extracts. Growth medium was phosphate-buffered AB minimal medium (Clark & Maaløe, 1967). Broth medium contained, per litre: 10 g Bactotryptone (Difco), 5 g yeast extract (Difco), 5 g NaCl, 1 g glucose. Solid media were prepared by adding 1.5% (w/v) agar (Difco) to AB or broth ingredients. Growth rates were determined by growing 20 ml cultures in 200 ml flasks with aeration by shaking at 37 °C. Growth was followed as optical density (OD) at 436 nm in an Eppendorf 1101M photometer. An OD₄₃₆ of 1.0 (1 cm light path) corresponds to approximately 3×10^8 cells ml⁻¹. Glucose or glycerol (0.2%) served as the carbon source. When necessary, amino acids were added to 40 mg l⁻¹. Purine bases were added to 15 mg l⁻¹, ribonucleosides to 30 mg l⁻¹. Nicotinic acid was added to 1 mg l⁻¹. Thiamin (1 mg l⁻¹) was added to all minimal media unless otherwise stated. Tetracycline was used at 5 mg l⁻¹. Cultures for enzyme analysis were grown at 37 °C with aeration by air bubbling. At an OD₄₃₆ of approximately 1 the cells were cooled and harvested by centrifugation. Following a wash in 0.9% saline, the cells were suspended in 50 mM-Tris/HCl, pH 7.8, at 50 times the original cell density. After homogenization by sonication and removal of debris by centrifugation, the cell extract was desalted by passage through a small column of Sephadex G25, equilibrated with 50 mM-Tris/HCl, pH 7.8.

Guanosine kinase assay. Specific activity is given as nmol min⁻¹ (mg protein)⁻¹. Protein content was determined by the Lowry method, with bovine serum albumin as the standard. Guanosine kinase activity was determined by a modification of the procedure described by Jochimsen *et al.* (1975). The assay contained, in a final volume of 0.1 ml: 70 mM-Tris/HCl, pH 7.8; 35 mM-KCl; 35 mM-MgCl₂; 1 mM-ATP; 0.5 mM (18.5 GBq mol⁻¹) [U-¹⁴C]guanosine; 10 mM-phosphoenolpyruvate; 1 µg pyruvate kinase; and guanosine kinase activity (0.2–1.0 nmol min⁻¹). Samples were removed at appropriate intervals and processed as described by Jochimsen *et al.* (1975).

Incorporation of [¹⁴C]guanosine into nucleic acids. At an OD₄₃₆ of 0.05, cells growing exponentially at 37 °C were supplemented with 10 µM-(40 GBq mol⁻¹) [8-¹⁴C]guanosine and allowed to incorporate radioactivity until an OD₄₃₆ of 1 was reached. Then the cells were treated with 0.5 M-trichloroacetic acid and the radioactivity incorporated into nucleic acids was determined as acid-insoluble material.

Determination of 5-aminoimidazole ribonucleoside 5'-monophosphate (AIR). The procedure of Bratton & Marshall (1939) was employed. In this procedure AIR, excreted from the cells as the ribonucleoside, is converted to a coloured diazo compound, with an absorption maximum at 500 nm.

Ribonucleoside triphosphate pool determinations. Cells were grown in a Tris-buffered medium containing 0.3 mM-P_i (Jensen *et al.*, 1979). After several generations of exponential growth, carrier-free ³²P_i (Forsøgscenter Risø, Denmark) was added at 1.22 TBq mol⁻¹. Following an additional two generations of growth, the nucleotides were extracted by addition of HCOOH to a final concentration of 0.33 M. Nucleotides were separated by two-dimensional thin-layer chromatography as described by Neuhaard *et al.* (1965).

Mutagenization. Cells growing exponentially in supplemented minimal medium were harvested, washed and resuspended in unsupplemented medium, placed under a UV lamp, irradiated to approximately 0.1% survival and grown overnight in minimal medium for phenotypic expression. Alternatively, the cells were infected with bacteriophage Muets62 by placing droplets of a phage lysate onto a lawn of recipient cells in soft agar. Cells from the infected area were cut out of the agar, inoculated in broth medium and grown overnight for phenotypic expression.

Transduction. P1vir lysates were prepared by the soft agar overlay technique and transduction was performed as described by Lennox (1955). Genetic markers were scored as follows: the *acrA* marker was scored on broth plates supplemented with acriflavine (Nakamura *et al.*, 1975); *adk* is a temperature-sensitive marker (Ray *et al.*, 1970); *apt* was scored on plates with adenine serving as purine source (Jochimsen *et al.*, 1975).

Table 1. *Bacterial strains used*

Strain	Genotype*	Source/construction/application
HO1	<i>metB rpsL relA spoT supF lamB purE deoD apt purL gsk-10</i>	This work, SØ446, UV mutagenesis
HO3	<i>metB rpsL relA spoT supF lamB purE deoD apt gsk-3</i>	This work, SØ446, UV mutagenesis
HO4	<i>metB rpsL relA spoT supF lamB purE deoD apt purF gsk-4</i>	This work, SØ446, UV mutagenesis
HO20	<i>metB rpsL relA spoT supF lamB purE deoD apt purM::Mucts62 gsk-9</i>	This work, SØ446, Mu mutagenesis
SØ1172	<i>metB rpsL relA spoT supF lamB purE deoD apt lon prs-1 gsk-2</i>	From SØ446, UV mutagenesis (Hove-Jensen & Nygaard, 1982)
AB2849	<i>aroC</i>	A. J. Pittard, through CGSC†
HO9	<i>metB rpsL relA spoT supF lamB purE deoD apt purM::ΔMucts62 gsk-9</i>	From HO20 by plating at 42 °C
HO48	<i>metB rpsL relA spoT supF lamB purE deoD apt lacZY</i>	SØ446, Lac ⁻ (Smith & Sadler, 1971)
HO54	<i>metB rpsL relA spoT supF lamB purE deoD apt lon prs-1 gsk-2 proC</i>	Hove-Jensen (1983)
HO55	<i>metB rpsL relA spoT supF lamB purE deoD apt proC</i>	P1(HO54) × HO48, Lac ⁺
HO105	<i>nadB argH thi</i>	P1(SØ003) × PA3306, Pur ⁺
HO419	<i>metB rpsL relA spoT supF lamB zbb-2419::Tn10‡</i>	This work, Tn10 insertion near <i>gsk</i>
N43	<i>ara lac galK malA xyl mtl rpsL acrA1</i>	Nakamura <i>et al.</i> (1975), via CGSC†
N2485	<i>adk-2 lac</i>	Nakamura <i>et al.</i> (1975)
ORF4/KL251	<i>lac⁺ purE⁺ apt⁺/thi metE trpE purE proC leu recA lacZ</i>	K. B. Low, through CGSC†
PA3306	<i>purL nadB argH thi</i>	Lavallé, through CGSC†
SØ003	<i>metB rpsL relA spoT supF lamB</i>	Defined wild-type strain, derivative of <i>E. coli</i> K-12 strain W6 (Bachmann, 1972)
SØ198	<i>metB rpsL relA spoT supF lamB guaA</i>	From SØ003, UV mutagenesis and penicillin counterselection
SØ199	<i>metB rpsL relA spoT supF lamB purE</i>	Jochimsen <i>et al.</i> (1975)
SØ236	<i>metB rpsL relA spoT supF lamB guaA thyA deoD</i>	From SØ198, K. Hammer (unpublished data)
SØ312	<i>metB rpsL relA spoT supF lamB purE deoD</i>	Jochimsen <i>et al.</i> (1975)
SØ405	<i>metB rpsL relA spoT supF lamB purB add deoD</i>	Jochimsen <i>et al.</i> (1975)
SØ408	<i>metB rpsL relA spoT supF lamB upp</i>	Hammer-Jespersen & Munch-Petersen (1973)
SØ445	<i>metB rpsL relA spoT supF lamB purE deoD gsk-1</i>	Jochimsen <i>et al.</i> (1975)
SØ446	<i>metB rpsL relA spoT supF lamB purE deoD apt</i>	Parental strain for mutagenization (Jochimsen <i>et al.</i> , 1975)
SØ490	<i>metB rpsL relA spoT supF lamB guaA thyA deoD deoB</i>	From SØ236, low thymine requiring
SØ1023	<i>nupG::Tn10 thi relA</i>	B. Mygind (unpublished data)
SØ1098	<i>metB rpsL relA spoT supF lamB deoD guaA</i>	P1(SØ003) × SØ236, Thy ⁺
SØ1628	<i>metB rpsL relA spoT supF lamB deoD guaA nupG::Tn10</i>	P1(SØ1023) × SØ1098, Tet ^R
SØ1704	<i>adk-2 lac zbb-2419::Tn10</i>	P1(HO419) × N2485, Tet ^R
SØ1726	<i>metB rpsL relA spoT supF lamB purE deoD adk-2 zbb-2419::Tn10</i>	P1(SØ1704) × SØ445, Tet ^R

* All the strains are F⁻ strains, except ORF4/KL251, which is an F⁺ strain, and AB2849 (F⁺).

† Obtained through the Coli Genetic Stock Center (CGSC).

‡ Nomenclature of Tn10 insertions follows the rules of Chumley *et al.* (1979).

Sexduction. Equal numbers of exponentially growing donor and recipient cells in minimal medium were mixed for 1 h at 37 °C and diluted samples plated on selective medium. Streptomycin (200 mg l⁻¹) was used for counterselection.

Isolation of a Tn10 insertion near the gsk gene. A bacteriophage P1 lysate was grown on the pool of randomly inserted Tn10 transposons [transposable genetic element coding for tetracycline resistance (Tet^R)] previously described (Hove-Jensen, 1983). This lysate was used to infect strain SØ199 (*purE*) with subsequent selection for Pur⁺Tet^R. Among a number of independent Tn10 insertions obtained and mapped, *zbb-2419::Tn10* (HO419) was used in this work.

Isolation of mutants able to utilize guanosine as sole purine source. The strain SØ446 was submitted to UV irradiation or bacteriophage Mu treatment as described above. Following phenotypic expression, 10⁸ cells were plated on glucose minimal medium with guanosine as the sole source of purine. Colonies were purified on the same medium; *pur*⁺ and *deoD*⁺ revertants were discarded.

Chemicals. All chemicals were commercially available and used without further treatment. [U-¹⁴C]Guanosine and [8-¹⁴C]guanosine were obtained from Amersham. Phosphoenolpyruvate and pyruvate kinase were from Boehringer Mannheim.

RESULTS

In vivo evidence for guanosine and inosine kinase activities

The guanine requirement of *E. coli* *gua* strains can be fulfilled not only by guanine, but also by guanosine. This is also true if purine nucleoside phosphorylase (encoded by *deoD*) is non-functional. As shown in Table 2, strain SØ490 (*guaA deoD*) grows almost normally with guanosine serving as source of guanine nucleotides, and its purine ribonucleoside triphosphate pools are almost normal. These results indicate that a pathway different from that involving purine nucleoside phosphorylase for the conversion of guanosine to GMP exists.

To demonstrate inosine utilization, we used a strain defective in inosine 5'-monophosphate synthesis (Fig. 1), SØ312 (*purE deoD*), which can be supplied by hypoxanthine or by inosine as shown in Table 2. However, inosine only poorly fulfils the requirement. If, instead, a strain defective in inosine 5'-monophosphate synthesis and also defective in AMP synthesis (Fig. 1), SØ405 (*purB deoD*) is used, inosine satisfies quite well the need for guanine nucleotides when adenine serves as adenine nucleotide source. Thus, there are only minor differences in the growth rate or in the pools of ATP and GTP, whether the strain is grown with hypoxanthine or inosine to supply the guanine nucleotides. In these experiments, histidine was added to prevent adenine from being converted to guanine nucleotides through the histidine pathway (Fig. 1). The results clearly demonstrate that pathways different from phosphorolysis of guanosine and inosine exist. These pathways depend on guanosine and inosine kinase activities.

Guanosine transport

To determine whether the guanosine transport system (*nupG*) was a growth-limiting factor in the utilization of guanosine, we compared the growth of the two strains SØ1098 (*guaA deoD*) and SØ1628 (*guaA deoD nupG*). Although the transport of guanosine was reduced in the *nupG* strain

Table 2. *In vivo evidence for guanosine and inosine kinase activities of E. coli*

Growth rates and ribonucleotide pools were determined as described in Methods. Values showed less than 10% variation in duplicate experiments.

Strain	Genotype	Addition to medium	Doubling time (min)	Nucleotide pool size [nmol (mg dry weight) ⁻¹]	
				ATP	GTP
SØ490	<i>guaA deoD</i>	Guanine	58	3.2	2.3
		Guanosine	68	3.0	1.2
SØ312	<i>purE deoD</i>	Hypoxanthine	46	6.0	2.9
		Inosine	200	5.7	0.7
SØ405	<i>purB deoD</i> *	Adenine, hypoxanthine, histidine	56	8.5	1.8
		Adenine, inosine, histidine	74	8.9	1.2

* This strain cannot grow with adenine as the sole purine source.

Table 3. *Growth rates of guanosine-utilizing mutants on different purine sources*

Growth medium was AB glucose supplemented with thiamin and the indicated purine source. Values indicate doubling time in min.

Strain	Purine source			
	Guanosine	Inosine	Guanine	Hypoxanthine
SØ446	> 600	220	> 250*	47
HO1	78	175	48	47
HO3	111	99	196	44
HO4	56	137	52	43
HO9†	86	100	60	44
SØ1172	58	89	> 600	82

* Balanced growth not obtained.

† HO9 is a temperature-resistant derivative of HO20 (Table 1).

Table 4. *Guanosine kinase activity and guanosine incorporation in guanosine-utilizing mutants*

Guanosine kinase activity was determined at the temperature indicated by the procedure described in Methods. Incorporation of guanosine into acid-insoluble material was performed as described in Methods.

Strain	Guanosine kinase activity [nmol min ⁻¹ (mg protein) ⁻¹]		Guanosine incorporation [nmol (mg dry weight) ⁻¹]
	37 °C	42 °C	
SØ446 <i>gsk</i> ⁺	5.0	0.4	10
HO1 <i>gsk</i> -10	5.0	2.3	125
HO3 <i>gsk</i> -3	6.8	8.6	135
HO4 <i>gsk</i> -4	6.6	6.9	130
HO9 <i>gsk</i> -9	8.5	8.3	105
SØ1172 <i>gsk</i> -2	5.7	2.9	60

as compared to the *nupG*⁺ strain (data not shown), there was no difference in growth rate between the two strains, when the guanosine concentration was varied from 7 to 100 µM.

Isolation and characterization of mutants with improved utilization of guanosine for nucleotide synthesis

The utilization of purine ribonucleosides is not very efficient by *purE deoD* strains of *E. coli*. Specifically, the utilization of guanosine is very poor. Besides guanosine kinase, the utilization of guanosine by *purE deoD* strains requires the participation of yet another enzyme, GMP reductase (Fig. 1). Although guanosine kinase and GMP reductase can be detected in extracts of *purE deoD* strains, growth does not occur with guanosine. Assuming that the limiting step under these conditions is the phosphorylation of guanosine, it should be possible to isolate mutants with increased guanosine kinase activity by plating mutagenized *pur deoD* cells on medium with guanosine serving as sole purine source as described in Methods. From this selection, the following mutants were isolated: HO1, HO3, HO4, HO20 and SØ1172 (Table 1). The growth rates of the guanosine-utilizing mutants supplemented with different purine sources were determined (Table 3). The results indicate that (i) the growth of HO1, HO4 and HO9 is improved not only on guanosine, but also on guanine, a compound that is metabolized without the participation of guanosine kinase, and (ii) growth on inosine is also increased although not to the same extent as on guanosine for strains HO1 and HO4, HO9 and SØ1172 (Table 3). Inosine is metabolized via guanosine kinase.

The mutants were assayed for guanosine kinase, and all had altered guanosine kinase activity (Table 4). Although at 37 °C there was either no difference or only minor differences between the parent and the mutants, when the enzyme was assayed at 42 °C the mutants showed a 6- to

Table 5. Genetic characterization of guanosine-utilizing mutants with secondary purine blocks

P1 transductions were performed as described in Methods, on medium containing glucose and the indicated supplements.

Expt no.	Recipient strain	Donor strain	Selected marker	Scored marker	Supplement(s)	Co-transduction frequency†
1	HO1 (<i>purL</i>)/F254*	HO105 (<i>nadB</i>)	Pur ⁺	Nad	Nicotinate	0.11 (318)
2	AB2849 (<i>aroC</i>)	HO4 (<i>purF</i>)	Aro ⁺	Pur	Hypoxanthine and thiamin	0.41 (370)
3	HO9 (<i>purM</i>)	SØ408 (<i>upp</i>)	Thiamin independence	Upp‡	Hypoxanthine	0.95 (60)

* The episome was transferred from ORF4/KL251 as described in Methods, by selection for *apt*⁺ on plates with adenine as the purine source.

† The number of transductants examined is given in parenthesis.

‡ The Upp phenotype was scored on plates containing 5-fluorouracil (2.5 mg l⁻¹). Cells that are *upp* are resistant, whereas *upp*⁺ cells are sensitive to 5-fluorouracil.

22-fold increase in specific activity. We also determined the incorporation of guanosine into nucleic acids at 37 °C, and found that the mutants had a 6- to 14-fold increase in incorporation (Table 4). From the enzyme and incorporation data we conclude that the mutants have alterations in the gene specifying guanosine kinase. These mutations result in enzymes that function more efficiently *in vivo*, and *in vitro* they appear more heat-stable.

Genetic mapping of the mutations causing altered guanosine kinase activity

To demonstrate that the mutations causing the alterations in guanosine kinase mapped in the *gsk* gene we isolated a Tn10 insertion (*zbb-2419* :: Tn10) between *gsk* and *purE*, which are closely linked markers (Jochimsen *et al.*, 1975). The *zbb-2419* :: Tn10 allele was then transduced into strain HO3 (*purE gsk-3*), resulting in a strain with the genotype *purE gsk-3 zbb-2419* :: Tn10. The presence of an altered guanosine kinase was confirmed by assay. A P1 lysate was then grown on this *purE gsk-3 zbb-2419* :: Tn10 strain and used to transduce strain SØ445 (*purE deoD gsk-1*) to tetracycline resistance. Of the 61 transductants analysed, 31% were able to use guanosine for GMP synthesis, indicating acquisition of a functional *gsk* gene. Moreover, all these transductants were able to use guanosine as sole purine source, indicating acquisition of the altered guanosine kinase. This was confirmed by assay of the enzyme in some of the transductants. Identical results were obtained for the *gsk-2* and *gsk-9* alleles. We therefore conclude that the lesions causing the altered guanosine kinases are, indeed, alleles of the *gsk* gene.

Mutants with secondary blocks

While working with the mutants described above, we noticed that the strains HO1, HO4 and HO9 could not be transduced to Pur⁺ by P1 transduction, indicating that the mutants in some way had become double purine auxotrophs. This was confirmed by introducing the episome F254 (*purE*⁺) into the strains. The purine lesions of strains HO1, HO4 and HO9 were characterized by identifying the purine pathway intermediates that the strains accumulated during purine starvation and by genetic mapping of the lesions. None of the mutants accumulated AIR as did the parental *purE* strain, suggesting mutations in genes specifying the purine enzymes catalysing the early steps of the pathway. All three strains accumulated phosphoribosyl pyrophosphate, but HO4 did so to a much greater extent than HO1 and HO9, characteristic of *purF* strains (Jensen *et al.*, 1979). Strain HO1 accumulated formylglycinamide ribonucleoside 5'-monophosphate and HO9 formylglycinamide ribonucleoside 5'-monophosphate (cf. Fig. 1), determined by the procedure of Houlberg *et al.* (1983). These results indicate that HO1 is defective in *purL*, HO4 in *purF* and HO9 in *purM*.

The secondary purine lesions in strains HO1, HO4 and HO9 were mapped by cotransduction with appropriate markers as shown in Table 5. The data indicate a location of the *pur* marker in strain HO1 close to *nadB*, consistent with *purL* (experiment no. 1). In strain HO4 the purine

Fig. 2. Map of the *proC*-*purE* region of the *E. coli* chromosome. The bar indicates the chromosome. Numbers below the bar are map units in min (Bachmann, 1983). *Tn10* indicates the *zbb-2419::Tn10* allele. Values above the arrows are percentage co-transduction frequencies based on the data from Table 6 and previously published data (Jochimsen *et al.*, 1975). Arrowheads point towards the unselected markers.

marker was closely linked to *aroC*, indicative of *purF* (experiment no. 2, Table 5). Finally the secondary *pur* marker in strain HO9 was shown to be *purM*. Strain HO9 required thiamin for growth (Fig. 1). By selection in transduction for thiamin independence, recombinants that had lost the secondary *pur* lesion were obtained. The data given in Table 5 (experiment no. 3) revealed that the mutation is very closely linked with *upp*, as is *purM*. It appears that the selection of guanosine-utilizing mutants has given rise to double mutants HO1 (*purL gsk-10*), HO4 (*purF gsk-4*), HO9 (*purM gsk-9*), and also SØ1172 (*prs gsk-2*). The latter strain has been previously characterized with respect to the defective phosphoribosylpyrophosphate synthetase, encoded by *prs* (Hove-Jensen & Nygaard, 1982; Hove-Jensen, 1983).

Genetic mapping of *gsk*

While studying the genetics of the *gsk* mutants, we found that the *gsk* gene was incorrectly placed on the current linkage map (Jochimsen *et al.*, 1975; Bachmann, 1983). Although we confirmed the linkage of *gsk* and *purE*, we noticed that *gsk* was located counter-clockwise of *purE* rather than clockwise. The previous clockwise assignment was based on two-factor crosses and on complementation data using various F-episomes. We have performed a more detailed genetic analysis of the *proC*-*purE* region including the markers *acrA*, *adk*, *apt* and *zbb-2419::Tn10*. The results obtained from three-factor crosses are given in Table 6 and the gene order found is shown in Fig. 2.

DISCUSSION

Guanosine can be converted to GMP by either of two pathways in *E. coli*: (i) phosphorolysis to guanine (and ribose 1-phosphate) followed by phosphoribosylation of the guanine moiety, or (ii) direct phosphorylation (Fig. 1). Both pathways appear to operate in wild-type *E. coli* cells when guanosine is taken up from the culture medium and metabolized (Jensen, 1978). It is not known by which pathway the guanosine nucleotides degraded inside the cells are salvaged. The various nucleotidases, most of which are ectoenzymes, degrade nucleoside monophosphates to the nucleosides. Recently an enzyme that degrades dGTP and GTP to deoxyguanosine and guanosine, respectively, has been purified and studied (Seto *et al.*, 1988). The physiological role of this enzyme is not known.

In the present study, we have investigated the guanosine kinase pathway. First it appears that the pathway is inefficient. Exogenous guanosine can be used when only GMP is needed, but

Table 6. *Genetic mapping of the gsk gene*

Bacteriophage P1 mediated transductions were conducted as described in Methods. The Tet^R marker of strain SØ1726 is the *zbb-2419::Tn10* allele (Table 1).

Recipient strain	Donor strain	Selected marker	Scored markers	No.
HO55 (<i>proC purE apt</i>)	N43 (<i>acrA</i>)	<i>pro</i> ⁺	<i>acrA</i> ⁺ <i>apt</i>	473
			<i>acrA</i> <i>apt</i>	13
			<i>acrA</i> <i>apt</i> ⁺	8
			<i>acrA</i> ⁺ <i>apt</i> ⁺	3
		<i>pur</i> ⁺	<i>acrA</i> ⁺ <i>apt</i>	199
			<i>acrA</i> ⁺ <i>apt</i> ⁺	6
			<i>acrA</i> <i>apt</i> ⁺	1
SØ1726 (Tet ^R <i>adk</i>)	SØ445 (<i>gsk-I</i>)	<i>adk</i> ⁺	<i>gsk</i> ⁺ Tet ^R	64
			<i>gsk</i> Tet ^R	137
			<i>gsk</i> Tet ^S	182
			<i>gsk</i> ⁺ Tet ^S	19
SØ1726 (Tet ^R <i>adk</i>)	SØ446 (<i>apt</i>)	<i>adk</i> ⁺	<i>apt</i> ⁺ Tet ^R	29
			<i>apt</i> Tet ^R	77
			<i>apt</i> ⁺ Tet ^S	22
			<i>apt</i> Tet ^S	78

cannot be used when there is an additional demand to supply AMP. The limiting process apparently is not guanosine transport. Rather the formation of GMP from guanosine most likely is growth limiting in *purE deoD* strains, but this is not the case in *guaA deoD* strains. The difference between the two strains is that ATP is abundant in the *guaA* strain. In contrast to the *purE* strain, the *de novo* synthesis of AMP is not impaired in the *guaA* strain. ATP is not only required by guanosine kinase for activity, but high levels stimulate the enzyme. As a consequence, only small amounts of GMP are formed in the *purE deoD* strain and conversion to AMP does not occur via the GMP reductase pathway (Fig. 1), because the induction of this conversion pathway requires a high level of guanine nucleotides. This appears to explain why *purE deoD* strains do not utilize guanosine as the sole purine source. Another consideration is that ATP stimulates the synthesis of GMP and also inhibits the conversion of GMP to AMP. Thus, the increased synthesis of GMP in *guaA deoD* strains solely furnishes the cell's demand for guanine nucleotides.

The fact that *E. coli* possesses guanosine kinase activity probably reflects that guanosine salvage occurs and that this only can be a supplement to the overall nucleotide synthesis. All the guanosine-utilizing mutants isolated showed altered guanosine kinase activity (Table 4). Preliminary kinetic investigations of guanosine kinase in cell-free extracts from the *gsk* mutants indicate that the kinetic properties are altered, the apparent K_m for guanosine being lowered. Besides the *gsk* mutation we identified mutations that caused deficiencies in one of the early enzymes of the purine biosynthetic pathway, catalysing the steps before the *purE*-encoded reaction. If we consider the synthesis of phosphoribosylpyrophosphate as an early step in purine biosynthesis (Fig. 1), strain SØ1172 belongs to this group of double mutants (Table 4). An exception to this is strain HO3. In this mutant, we found no evidence for additional mutations. However, the growth of this strain on guanosine is reduced compared to the double mutants (Table 3). The additional defects in the *de novo* purine pathway are apparently advantageous.

When *purE* strains are starved for purines they accumulate AIR. Because this compound in fact is a purine analogue, it appears to inhibit guanosine metabolism. The guanosine-utilizing mutants isolated by Livshits & Sukhodolets (1973b) were obtained in a *purD deoD* genetic background. This proves that accumulation of AIR during purine starvation is not a prerequisite for the isolation of guanosine-utilizing mutants. Rather, AIR further stresses the cells and gives rise to mutants synthesizing more efficient enzymes. As can be seen in Table 3 the guanosine-utilizing mutants HO1, HO4 and HO9 (with additional defects in a *pur* gene) also grew better on guanine than did HO3, which harbours a *gsk* mutation alone. This might indicate

that the GMP reductase pathway is inhibited by AIR which accumulates in *purE* strains when starved for purines.

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