Genetic Separation of Purine Transport from Phosphoribosyltransferase Activity in *Salmonella typhimurium*

By CHARLES E. BENSON,^{1,2} DAVID L. HORNICK¹
AND JOSEPH S. GOTS^{1*}

¹ Department of Microbiology, School of Medicine and ² Department of Medical Technology, School of Allied Medical Professions, University of Pennsylvania, Philadelphia, Pennsylvania, 19104, U.S.A.

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Two mutants of *Salmonella typhimurium* were isolated which differ from their respective parental strains in their growth responses to guanine and xanthine. Both mutants had purine phosphoribosyltransferase activities similar to their parental strains. One mutant, CB-3, had a lower guanine uptake rate apparently caused by a genetic lesion in a specific gene (designated *guaP*) responsible for facilitating the transport of guanine. This gene mapped at 3.5 min in the sequence *azi-guaP-nadC*. The second mutant, GP103, had a purine carrier molecule with altered specificity, as demonstrated by a competition between hypoxanthine and xanthine for uptake.

INTRODUCTION

The transport of exogenous purine bases into microbial cells has been described as a group translocation mediated by membrane-bound phosphoribosyltransferases (PRTs) (Berlin & Oliver, 1975; Hochstadt-Ozer & Stadtman, 1971*a*; Jackman & Hochstadt, 1976) during which each base is converted to its corresponding ribonucleotide by 5-phosphoribosyl transfer from 5-phosphoribosyl-1-pyrophosphate. Evidence supporting this conclusion includes the observation that variations in substrates and inhibitors produced similar effects on transport and PRT activity (Zylka & Plagemann, 1975), and that the loss of a PRT activity was always followed by specific defects in purine uptake (Hochstadt-Ozer & Stadtman, 1971*a, b*).

Several reports (Pickering & Woods, 1972; Roy-Burman & Visser, 1975; Thakar & Kalle, 1968) suggest that purine transport, defined as the movement of bases across the membrane, is a function separate from phosphoribosylation. There are differences between the transport K_m for a specific purine base and the K_m of the corresponding PRT (Thakar & Kalle, 1968). Dissimilarities in the effects of various inhibitors on transport and PRT activity have also been noted (Berlin, 1970; Berlin & Oliver, 1975; Roy-Burman & Visser, 1975; Thakar & Kalle, 1968). Furthermore, transport-deficient mutants with normal PRT activity have been isolated (Roy-Burman & Visser, 1975; Thakar & Kalle, 1968), and in mammalian cells PRT-deficient (or KCN-treated) cells have been shown to accumulate labelled purines under appropriate conditions (Alford & Barnes, 1976; Zylka & Plagemann, 1975). Such results imply that an additional component, distinct from the PRTs, may be involved in the transport process.

This paper describes the isolation and characterization of two *Salmonella typhimurium* strains with defects in purine uptake that are functionally and genetically distinct from the activity of the corresponding PRTs.

Table 1. *Bacterial strains used*

Strain	Description*	Source/reference
<i>Salmonella typhimurium</i>		
LT-2	Wild-type	M. Demerec
AB47	$\Delta(\text{proAB47-gpt})$	M. Demerec
SL751	<i>proA46 purC7 purI509 ilvA405 strA rha461</i>	B. Stocker
CB-3	as SL751, but <i>guaP</i>	This paper
TM445	as SL751, but <i>aroP505 hpt guaC</i>	Chou & Martin (1972)
GP103	as TM445, but with Xar phenotype	This paper
PM553	$\Delta(\text{proAB47-gpt}) \text{ leu-798 fol-101}$	P. Margolin
SM16	$\Delta(\text{aroP-aceB-nadC})$	Langley & Guest (1974)
Pan5	<i>pan-5</i>	Langley & Guest (1974)
0002	<i>aziA gal hisA metA nml rha trpB</i>	Langley & Guest (1974)
KP4065	<i>purA deoD add</i>	J. Hoffmeyer
JF12	as SL751, but <i>nadC19</i>	Foster & Moat (1978)
SA464	HfrK1-2 (O- <i>purE...cysE</i>) cw; <i>serA13 rfa-3058</i>	Sanderson <i>et al.</i> (1972)
SA955	HfrK20 (O- <i>purE...gal</i>) ccw; <i>serA13 rfa-3058</i>	Sanderson <i>et al.</i> (1972)
<i>Escherichia coli</i> †		
HΔ10	$\Delta(\text{nadC-aroP-ace})$	Langley & Guest (1977)
KΔ17	$\Delta(\text{nadC-aroP-ace})$	Langley & Guest (1977)
KΔ22	$\Delta(\text{nadC-aroP-ace})$	Langley & Guest (1977)
KΔ27	$\Delta(\text{nadC-aroP})$	Langley & Guest (1977)
HΔ3	$\Delta(\text{nadC-aroP})$	Langley & Guest (1977)

* Gene symbols and abbreviations are described by Sanderson & Hartman (1978).

† Strains described by Langley & Guest (1977, 1978). H parent: Hfr; *thi-1 azi-7 relA1*. K parent: F⁻; *metB relA1 thyA56 tsx-87 pps azi*.

METHODS

Bacterial strains. The strains used in this study were all derivatives of *Salmonella typhimurium* LT-2 or *Escherichia coli* K-12 (Table 1). Transductions were performed as plate crosses using 0.1 ml of P22 transducing phage and 0.1 ml of a washed recipient broth culture. Preparation of P22 transducing phage lysates and their assay followed standard procedures (Ely *et al.*, 1974). Conjugation with Hfr or episome-carrying strains was achieved by plate crosses or by concentrating donor and recipient strains to a pellet and interrupting the mating by vigorous vortex and pipette agitation. P1 transduction of *Escherichia coli* strains was performed according to the technique of Rosner (1972).

Media and culture techniques. The minimal salts medium (ME) of Vogel & Bonner (1956) was used with glucose (0.2%) as the carbon source. When necessary, amino acid requirements were satisfied with either 40 µg ml⁻¹ of the appropriate amino acid or 0.1% (w/v) casein hydrolysate. Purine supplements were 20 µg ml⁻¹ unless otherwise indicated. Quantitative growth responses were monitored in liquid media with a Klett-Summerson photocolormeter using the green filter (no. 54). Tube cultures (5.0 ml) were aerated on a roller drum apparatus and large broth cultures (100 or 200 ml) were incubated overnight in 1 l culture flasks on a rotary shaker water-bath (New Brunswick). All cultures were incubated at 37°C.

Chemicals. The tetrasodium salt of 5-phosphoribosyl-1-pyrophosphate was purchased from Sigma. [8-¹⁴C]Hypoxanthine (48.6 mCi mmol⁻¹, 1.798 GBq mmol⁻¹), [8-¹⁴C]adenine (53.5 mCi mmol⁻¹, 1.980 GBq mmol⁻¹), [8-¹⁴C]guanine (5.41 mCi mmol⁻¹, 200.2 MBq mmol⁻¹) and [8-¹⁴C]guanosine (49.2 mCi mmol⁻¹, 1.820 GBq mmol⁻¹) were obtained from New England Nuclear. [2-¹⁴C]Xanthine (48 mCi mmol⁻¹, 1.78 GBq mmol⁻¹) was purchased from Schwarz Mann. The scintillation fluid used in the Packard Tri-Carb scintillation counter was Omnifluor (New England Nuclear) dissolved in toluene (Baker, analytical grade). *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was obtained from Sigma.

Extracts and enzyme assays. Cultures were grown in 100 to 200 ml ME with appropriate supplements. Cell-free extracts were prepared and assayed for purine phosphoribosyltransferases and GMP reductase as described previously (Benson *et al.*, 1971; Gots *et al.*, 1972). Protein concentrations were determined by the Lowry method with bovine serum albumin as the standard.

In vitro competition with guanine-xanthine phosphoribosyltransferase (GPRT). The conversion of a ¹⁴C-labelled purine (hypoxanthine or xanthine) to its ribonucleotide form was measured in the absence and presence of an unlabelled competing purine. The concentration of the labelled purine was 50 nmol per 0.5 ml reaction mixture in a series of 12 tubes, while the concentration of the competing purine ranged from 2 to 100 nmol per 0.5 ml reaction mixture. The mixture was incubated at 37°C for 5 min and then the

reaction was terminated by placing the entire set of tubes in a boiling water bath for 2 min. The amount of nucleotide formed during incubation was established by thin-layer chromatography as described previously (Gots *et al.*, 1972).

Uptake of labelled purines. Overnight cultures were diluted 1:5 in ME medium supplemented with the appropriate purine base and amino acids and were grown for 1.5 h at 37 °C. Cultures were harvested by centrifugation and diluted in ME medium without glucose to an absorbance of 0.25 at 540 nm on a Zeiss spectrophotometer (equivalent to 60 µg dry wt ml⁻¹). Diluted cells were starved by stationary incubation at 37 °C for 1.5 h. At this time, 0.2 % glucose and the appropriate ¹⁴C-labelled purine base or nucleoside (0.05 µmol ml⁻¹, 0.05 µCi ml⁻¹) were added and 1 ml samples were collected every 20 s for 2 min on membrane filters (0.45 µm pore size, Gelman Instrument Co.). Each filter was washed immediately in 10 ml ME medium (without glucose) containing unlabelled purine base or nucleoside (50 µg ml⁻¹). Filters were dried and their radioactivities were determined in a Packard Tri-Carb scintillation counter.

RESULTS

Isolation of mutants

Cells were harvested by centrifugation from a 5 ml overnight nutrient broth culture of a *Salmonella typhimurium* purine auxotroph (strain SL751), washed once with 0.1 M-citrate buffer pH 5.5 and resuspended in 5 ml of the same buffer containing 150 µg NG ml⁻¹. The bacterial suspension was incubated for 1 h in a stationary water-bath at 37 °C. The cells were then collected by centrifugation, washed once with 0.1 M-phosphate buffer pH 7.4 and suspended in 100 ml ME supplemented with casein hydrolysate (0.1 %), uridine and guanine (each at 20 µg ml⁻¹ final concentration). The flask was incubated in a rotary shaker water-bath for 4 h at 37 °C, then ampicillin (40 µg ml⁻¹) was added to the culture and incubation was continued overnight at 37 °C with agitation. The cells were collected by centrifugation, washed four times with prewarmed 0.85 % (w/v) NaCl and the entire cell pellet was plated on four ME/glucose plates supplemented with 2 µg hypoxanthine ml⁻¹ and 40 µg guanine ml⁻¹. Mutants defective in guanine uptake or metabolism would appear as small colonies on this medium as their growth would be dependent on the hypoxanthine concentration. One mutant, designated CB-3, was selected for growth studies in defined liquid media to compare its phenotypic growth pattern with that of the parent strain. The generation time of mutant CB-3 with guanine or xanthine was four to five times higher than that of the parent (168 compared with 42 min), consistent with a defective guanine-xanthine phosphoribosyltransferase (GPRT) phenotype (Gots *et al.*, 1972).

A second mutant (GP103) with a defective GPRT phenotype was isolated from *S. typhimurium* strain TM445. As indicated in Table 1, strain TM445 has a double block (*purI* and *purC*) in the *de novo* biosynthesis of purines, is hypoxanthine (H)PRT deficient (*hpt*) and cannot convert GMP to IMP (*guaC*). The purine requirement may be satisfied with adenine via adenine (A)PRT or hypoxanthine via GPRT (Chou & Martin, 1972). When strain TM445 was plated on ME/glucose agar supplemented with casein hydrolysate and hypoxanthine, a zone of inhibition occurred around a paper disc saturated with guanine or xanthine. The inhibition is the result of competition between the natural substrate and hypoxanthine for GPRT. Colonies occasionally appeared within this zone of inhibition and one (designated GP103) was isolated for further characterization. Possible reversion to *guaC*⁺ was ruled out by confirming the absence of GMP reductase. Mutant GP103 had a generation time two- to fourfold greater than that of its parent, TM445, when grown on a defined medium supplemented with xanthine and hypoxanthine (each 20 µg ml⁻¹). These phenotypic characteristics may represent a change in substrate specificity of GPRT analogous to a previously described mutation in HPRT (Benson & Gots, 1975; Berlin & Oliver, 1975) or an altered purine uptake mechanism. The generation time of each mutant when grown in a hypoxanthine-supplemented medium was comparable with that of its respective parent strain. Thus, the phenotype relative to hypoxanthine uptake and metabolism appeared to be unaltered.

Table 2. *Purine phosphoribosyltransferase activities*

In all cases there were no significant differences in PRT activities with adenine (results not shown).

Strain	Relevant genotype	PRT activity (nmol nucleotide formed min ⁻¹)		
		Guanine	Hypoxanthine	Xanthine
LT-2		18.2	28.0	24.1
AB47	<i>gpt</i>	0.18	21.2	0.36
SL751		23.2	25.8	ND
CB-3	<i>guaP</i>	18.1	37.9	31.9
CB-3/F'128*	<i>guaP/gpt</i> ⁺	32.3	33.4	31.2
TM445	<i>hpt</i>	17.6	10.2	27.2
GP103	<i>hpt Xar</i>	12.9	10.4	24.7

ND, Not determined.

* F'128 is an F' episome carrying *pro*⁺ *gpt*⁺ (Low, 1972).

Table 3. *Uptake of guanine and guanosine*

Uptake was measured as described in Methods. The lower activities of the parent strain, SL751, compared with the prototroph were reproducible. All values were derived from the calculated uptake of ¹⁴C-labelled purine in 2 min; uptake was linear with time during this period. The results are expressed as a percentage of the uptake by the wild-type control, which was 400 pmol guanine (2 min)⁻¹ and 488 pmol guanosine (2 min)⁻¹.

Strain	Uptake (% of control)	
	Guanine	Guanosine
LT-2	100	100
AB47	18	25
SL751	73	81
CB-3	34	39
CB-3/F'128	46	56

Phosphoribosyltransferase studies

The phenotype of mutants CB-3 and GP103 could be explained by alterations in the respective PRT activities; however, no differences were found between parent and mutant in either case (Table 2). Genetic complementation of the GPRT gene (*gpt*) in CB-3 by the F'128 episome (*pro*⁺ *gpt*⁺) resulted in the expected gene dosage effect on GPRT activities, but did not improve the growth of the mutant on guanine. The PRT activities for all purine substrates were comparable between the two mutants (CB-3 and GP103) and their respective parent strains (SL751 and TM445). Thus, the different growth phenotypes were independent of the PRT activities, leading to the consideration that the substrate specificity of GPRT had been altered toward a greater affinity for hypoxanthine than guanine or xanthine. This possibility was easily investigated with strains TM445 and GP103 since HPRT was absent and a substrate competition of GPRT for hypoxanthine and xanthine could be measured with crude cell-free extracts. In both instances, the conversion of ¹⁴C-labelled hypoxanthine to IMP was significantly inhibited by the unlabelled xanthine, while conversion of xanthine to XMP was unaffected by hypoxanthine. Thus, the substrate specificity of GPRT in GP103 was similar to the parental strain.

Uptake of purine bases and nucleosides

An alternative explanation of the mutant phenotypes implicated a specific purine transport system in bacteria. The uptake of ¹⁴C-labelled purine bases and nucleosides was investigated using intact, resting cells of the mutant strain CB-3 and its parent SL751 (Table 3). The parent strain SL751 was less active in the uptake of guanine and guanosine than the original prototrophic strain, LT-2. This could indicate a membrane or transport

Table 4. *Competitive uptake studies*

Uptake was determined as described in Methods. Competition was measured by adding unlabelled purine ($0.1 \mu\text{mol ml}^{-1}$) with the labelled substrate. Results in parentheses show the uptake as a percentage of that in the control without addition.

^{14}C -labelled substrate	Unlabelled addition	Uptake [pmol (2 min)^{-1}]	
		Strain TM445	Strain GP103
Hypoxanthine	None	236	217
Hypoxanthine	Xanthine	76 (32 %)	209 (96 %)
Xanthine	None	113	62
Xanthine	Hypoxanthine	145 (128 %)	39 (63 %)

Table 5. *Cotransduction frequencies*

Transductions were performed as described in Methods. Cotransduction frequency is measured as the percentage of unselected marker scored among the selected transductants. The actual numbers of colonies examined are shown in parentheses.

Donor	Recipient	Selected marker	Unselected marker	Cotransduction (%)
CB-3	JF12	<i>nadC</i> ⁺	<i>guaP</i>	48 (21/43)
0002	CB-3	<i>guaP</i> ⁺	<i>aziA</i>	28 (14/50)
PM553	CB-3	<i>guaP</i> ⁺	<i>leu</i>	< 2 (0/50)
SM16	CB-3	<i>guaP</i> ⁺	<i>ace</i>	< 1 (0/100)
Pan5	CB-3	<i>guaP</i> ⁺	<i>pan</i>	< 2 (0/50)
KP4065	CB-3	<i>guaP</i> ⁺	<i>deoD</i>	< 2 (0/50)

defect which lowered the rate of purine uptake. This postulated defect apparently does not cause a significant change in the growth of the strain as determined by generation time measurements. However, mutant CB-3 was 50 % less active than strain SL751 in guanine and guanosine uptake and showed only one-third of the original wild-type activity. Although GPRT is important in the uptake of both guanine and guanosine, as shown by the results obtained with the *gpt* strain (AB47), the *gpt* merodiploids of CB-3/F'128 were still transport deficient. Thus, the defective uptake in CB-3 appears to be genetically distinct from *gpt*.

Analogous studies, performed with mutant strain GP103 and its parent TM445 indicated that both strains efficiently take up hypoxanthine and xanthine. However, in competitive uptake studies (Table 4), GP103 showed a reversal of the parental uptake specificity. The uptake of [^{14}C]hypoxanthine, normally inhibited by unlabelled xanthine, was unaffected by xanthine addition. Unlabelled hypoxanthine had no effect on [^{14}C]xanthine uptake by TM445, but inhibited the uptake of xanthine by strain GP103. This reversal in the uptake specificities may explain the ability of strain GP103 to continue utilizing hypoxanthine for growth in the presence of xanthine.

Mapping the genetic lesion in mutant CB-3

The phenotypic characteristic of strain CB-3 is poor growth on guanine (generation time three- to fourfold longer than for strain SL751), apparently due to a defect in a purine transport system. This transport defect is independent of the GPRT gene since PRT activity is normal in CB-3, and the F'128 episome, which carries the *gpt*⁺ gene, increases PRT activity for guanine without altering the transport deficiency. These two findings imply a genetic lesion in mutant CB-3 distinct from *gpt*. We designate this guanine-xanthine uptake defect as resulting from a mutation in the gene *guaP*. Conjugational studies with a series of *Salmonella* Hfr strains indicated a linkage of the GuaP phenotype with *ilv* and *pro*. A gradient of transmission model was constructed from results obtained in crosses with donor strains SA464 and SA955 (Sanderson *et al.*, 1972) placing *guaP* between 0 and 5 min

Table 6. *Uptake of [¹⁴C]guanine by E. coli strains*

Uptake of [¹⁴C]guanine was determined over a 2 min period as described in Methods. Results are expressed as a percentage of the uptake by a prototrophic *E. coli* K-12 control strain, which was 600 pmol (2 min)⁻¹. Ace⁺Nad⁺ transductants, obtained by P1 transduction of the designated mutants, were also tested.

Strain designation	Uptake (% of control)	
	Mutant	Ace ⁺ Nad ⁺ transductant
HΔ10	98.5	
HΔ3	28.7	97.4
KΔ22	15.0	59.0
KΔ17	15.0	
KΔ27	27.0	

on the *Salmonella* chromosome. Cotransduction studies (Table 5) with known genes in this region (Sanderson & Hartman, 1978) indicate the order, *azi-guaP-nadC*.

Detection and mapping of guaP in Escherichia coli

Langley and Guest (1977) isolated a series of deletion mutants in their studies of gene-enzyme relationships of 2-oxo-acid dehydrogenase in *E. coli*. The deletions in several of these mutants encompassed the region proposed by us to include the *guaP* locus. J. R. Guest generously supplied us with representative strains which we examined for the GuaP phenotype (Table 6). All strains except HΔ10 were deficient in guanine uptake and hence include *guaP* in their deletion. The deletion was corrected in two of these strains (KΔ22 and HΔ3) by P1 transduction with simultaneous selection for Nad⁺ and Ace⁺. These derivatives showed partial to full restoration of guanine uptake activity (Table 6). These results, in comparison with the proposed lengths of the deletion mutations (Langley & Guest, 1977, 1978), are consistent with the proposed order *aziA-guaP-nadC*.

DISCUSSION

We have described two mutants of *S. typhimurium* with alterations in guanine uptake. These lesions appear to be distinct and separate from the specific purine phosphoribosyl-transferases. The poor growth of strain CB-3 on guanine and xanthine mimics a PRT phenotype (Gots *et al.*, 1972) yet its PRT activities are unaltered and the defective uptake phenotype is not corrected when a plasmid carrying *gpt*⁺ is introduced. We have designated this defective guanine uptake phenotype GuaP. This provides genetic evidence that purine transport is mediated by a specific moiety separate from PRT. Burton (1977) suggested that uptake of purines is mediated by a transport system and that uptake is facilitated if the intracellular base is removed by a PRT. Our model completes this interpretation and extends the data by indicating a specificity in the transport system and the purine to be transported. The second mutant, GP103, could possess an altered carrier protein. The specificity of the uptake defect implies that at least one, and perhaps two or more, carrier proteins might be present for adenine and hypoxanthine. We propose, therefore, that the carrier protein has an integral association with the inner membrane of the cell, and functions as the initial recognition and entrance site for specific purine bases. Thus, purine metabolism could be altered via a change in the transport system or in the ribosylphosphorylation system (Berlin & Stadtman, 1966). This paper presents data to support this conclusion. The location of the *guaP* lesion was determined by the construction of a conjugational gradient of transmission graph and then pinpointed by cotransduction analysis. Studies with the deletion mutants of Langley & Guest (1977) indicated that the purine uptake mechanism is also present in *E. coli* and provided additional genetic data to confirm the gene sequence *aziA-guaP-nadC*.

Major questions remain concerning purine uptake, the nature of the carrier or transport system and the specificity of the system. While genetic and biochemical studies (Gots *et al.*, 1972; Hochstadt-Ozer & Stadtman, 1971*b*; Roy-Burman & Visser, 1975) have distinguished three PRTs in enteric bacteria (guanine-xanthine, hypoxanthine and adenine PRT) this paper presents the first genetic evidence of a specific purine transport system. Specific transport of guanine was first described by Thakar & Kalle (1968). Further study of purine transport should include the isolation of adenine and hypoxanthine transport-deficient strains.

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