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Utilization of 2,6-Diaminopurine by *Salmonella typhimurium*

BRUCE B. GARBER AND JOSEPH S. GOTS*

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

The pathway for the utilization of 2,6-diaminopurine (DAP) as an exogenous purine source in *Salmonella typhimurium* was examined. In strains able to use DAP as a purine source, mutant derivatives lacking either purine nucleoside phosphorylase or adenosine deaminase activity lost the ability to do so. The implied pathway of DAP utilization was via its conversion to DAP ribonucleoside by purine nucleoside phosphorylase, followed by deamination to guanosine by adenosine deaminase. Guanosine can then enter the established purine salvage pathways. In the course of defining this pathway, purine auxotrophs able to utilize DAP as sole purine source were isolated and partially characterized. These mutants fell into several classes, including (i) strains that only required an exogenous source of guanine nucleotides (e.g., *guaA* and *guaB* strains); (ii) strains that had a *purF* genetic lesion (i.e., were defective in α-5-phosphoribosyl 1-pyrophosphate amidotransferase activity); and (iii) strains that had constitutive levels of purine nucleoside phosphorylase. Selection among purine auxotrophs blocked in the de novo synthesis of inosine 5'-monophosphate, for efficient growth on DAP as sole source of purine nucleotides, readily yielded mutants which were defective in the regulation of their deoxyribonucleoside-catabolizing enzymes (e.g., *deoR* mutants).

The metabolism of 2,6-diaminopurine (DAP) has been investigated in several different systems. Early studies revealed that DAP could, under certain conditions, function as either a bacterial growth factor or as an inhibitor of growth (2, 23). The inhibition of growth due to DAP was suggested to be secondary to the conversion of DAP to inhibitory analogs of adenine-containing cofactors such as ATP, NAD, and flavin adenine dinucleotide (2, 26, 37), and to feedback inhibition by a DAP-derivative on de novo purine biosynthesis (14). Resistance to inhibitory concentrations of DAP was correlated with the inability to convert DAP to its nucleoside 5'-monophosphate form, due to a deficiency or alteration of adenine phosphoribosyltransferase activity (21, 22, 24, 32). DAP was found to act as a competitive inhibitor of adenine phosphoribosyltransferase (23), and this is in accord with the finding that growth inhibition by high concentrations of DAP could be reversed by adenine (11, 17). With regard to the utilization of DAP as an exogenous purine source, incorporation studies revealed that DAP was a precursor of both polynucleotide guanine and, to a lesser extent, polynucleotide adenine in bacteria (2). Guanine-requiring mutants that were blocked in the conversion of IMP to GMP, but able to synthesize AMP de novo, could readily utilize DAP as their sole exogenous purine source (1, 10, 23). However, purine auxotrophs blocked in the de novo synthesis of IMP, which required an exogenous purine able to supply both the adenine and guanine nucleotide pools, could satisfy their purine requirement with any natural purine (e.g., adenine, guanine, hypoxanthine, xanthine, and their derivatives) but not with DAP (1, 10). This paradox, i.e., that guanine could serve as a source of both guanine and adenine derivatives, whereas DAP apparently could serve only as a source of guanine derivatives, was examined by Demain and Shigeura (10, 33). They concluded that purine auxotrophs blocked in the synthesis of IMP failed to grow with DAP as sole purine source because, in addition to deamination of DAP to guanine (or DAP riboside to guanosine), DAP was converted to its nucleoside 5'-monophosphate via adenine phosphoribosyltransferase. Furthermore, DAP riboside monophosphate or a coenzyme derivative was probably the biologically inhibitory form of DAP. They also concluded that the mutants blocked in the conversion of IMP to GMP can grow on DAP because they are deficient in adenine phosphoribosyltransferase activity, due to feedback inhibition or repression by AMP or a derivative, and thus can carry out the deamination reaction predominantly.

Other studies pertinent to the present investigation indicate that, in mammalian systems, DAP can be converted to DAP ribonucleoside by purine nucleoside phosphorylase (25). Stud-

ies in *Salmonella* revealed that there is no adenase enzyme (31) and that adenosine deaminase is not inducible (31).

Our objectives in this investigation included (i) determination of the pathway whereby *Salmonella typhimurium* could utilize DAP as a source of guanine compounds, and (ii) determination of the reason(s) why purine auxotrophs blocked in the de novo synthesis of IMP could not use DAP as a sole exogenous source of purine compounds. Our approach involved the use of mutant strains defective in the synthesis, salvage, and interconversion of purines, as well as the isolation and characterization of mutant derivatives of purine auxotrophs that were able to utilize DAP as an effective source of all purines. In addition, we hoped that this study would shed some light on the regulation of the purine salvage and interconversion enzymes by yielding regulatory mutants.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study are derivatives of *S. typhimurium* LT2 or LT7 and are described in Table 1.

Chemicals. 6-Diazo-5-oxo-L-norleucine (DON) was obtained through the courtesy of H. Dion, Parke Davis and Co. (Detroit, Mich.). DAP and all other compounds were obtained from various commercial sources.

Media and culture techniques. The minimal salts medium was medium E described by Vogel and Bonner (35) with 0.2% glucose as the carbon source. This base medium was used for all growth studies and for all purine nucleoside phosphorylase and thymidine phosphorylase assays. For the cytidine deaminase assays, the base medium was that described by Berkowitz et al. (5) with 0.5% glycerol instead of lactose as the carbon source. Amino acid requirements were fulfilled by the appropriate amino acid at a concentration of 50 μg/ml except where otherwise stated, and thiamine (1.0 μg/ml) was routinely added to all growth media. All purine compounds (e.g., DAP, hypoxanthine, and adenine) were added to a final concentration of 50 μg/ml. Tube cultures were aerated in a roller drum apparatus, and large cultures were aerated on a rotary shaker water bath, at 37°C. Large cultures were inoculated from mid-log phase nutrient broth seed cultures, using 0.1 to 0.2 ml of seed culture per 100 ml of medium, and were incubated for 12 h for all enzyme assays. Culture turbidity was measured with a Klett-Summerson photocolorimeter with a green (no. 54) filter. DON was maintained in powdered form stored at −75°C until ready for use; then a solution of 1.0 mg/ml was made, filter sterilized, and added to the concentration specified in the individual experiments.

Preparation of cell extracts. All operations were carried out at 4°C. A 100-ml culture was harvested by centrifugation at 8,700 × *g* for 10 min, washed two times in 0.9% NaCl, and then suspended in 1.0 to 2.0 ml of sonication buffer (0.1 M Tris-hydrochloride, pH 7.7, and 2.0 mM EDTA). The concentrated cells were disrupted with a Branson sonifier for 30 s at 80 W, and the lysates were centrifuged at 27,000 × *g* for 1 h. For all purine nucleoside phosphorylase and thymidine

TABLE 1. *Bacterial Strains*

Strain	Genotype	Origin of source
LT2	Wild type	Laboratory stock collection
C7	*purC7*	Laboratory stock collection
B12	*purB12*	Laboratory stock collection
GP66	*purE66* Δ(*proAB-gpt*)*47*	Laboratory stock collection
A155	*purA155*	Laboratory stock collection
F183	*purF183*	Laboratory stock collection
H190	*purH190*	Laboratory stock collection
G310	*purG310*	Laboratory stock collection
D312	*purD312*	Laboratory stock collection
I314	*purI314*	Laboratory stock collection
D343	*purHD343*	Laboratory stock collection
J345	*purJ345*	Laboratory stock collection
GuaB5	*guaB5*	Laboratory stock collection
GuaB5 pup	*guaB5 deoD302*	Laboratory stock collection
KP4040-A	*add*	*purA*$^+$ revertant of KP4040 (19)
SL751	*proA46 purC7 purI590 ilvA405 rha-461 flaA56 strA*	B. Stocker
GP67	As GP66, plus *purF598*	This paper
GP67/F'128	GP67/F' *pro*$^+$ *lac*$^+$ *gpt*$^+$	This paper
GP66/F'128	GP66/F' *pro*$^+$ *lac*$^+$ *gpt*$^+$	This paper
GP70	*purG310 deoR301*	This paper
GP71	*purG310 deoR302*	This paper
GP72	As D343 plus *deoR303*	This paper
GP73	As SL751 but DPU[a]	This paper
GP88	As SL751 plus *deoR304*	This paper

[a] DPU, Unclassified DAP-utilizing mutant.

phosphorylase assays, the supernatants were dialyzed for 16 h against 500 volumes of 19 mM Tris-hydrochloride buffer (pH 7.6) containing 50 μM EDTA and 2.0 mM mercaptoethanol. For the cytidine deaminase assay the extracts were similarly dialyzed against the same buffer without mercaptoethanol.

Enzyme assays. Purine nucleoside phosphorylase was assayed by the coupled xanthine oxidase method of Kalckar (20). One milliliter of reaction mixture contained deoxyinosine (2.0 μmol), potassium phosphate buffer, pH 7.1 (150 μmol), and xanthine oxidase (0.063 enzyme units). The increase in absorbance, due to the formation of uric acid, was followed at 293 nm in a Gilford-modified Beckman spectrophotometer using a continuously recording strip chart. An extinction coefficient of 12.0 was used in the calculation of specific activity. Specific activity is defined as the number of nanomoles of uric acid formed per minute per milligram of protein at 37°C.

Thymidine phosphorylase was assayed as described by Hoffee (18). Specific activity is defined as the number of nanomoles of thymine formed per minute per milligram of protein at 37°C.

Cytidine deaminase was assayed as described by Hammer-Jespersen et al. (15). GMP reductase was measured as described by Benson et al. (3), and adenosine deaminase was determined as described by Remy and Love (31).

Protein determination. Protein concentrations were determined by the method of Lowry et al. (28), using bovine serum albumin as the standard.

Isolation of mutants able to utilize DAP. Two similar procedures were used. In the first, 10 ml of nutrient broth was inoculated with a single colony of a purine auxotroph. The cells were grown to mid-log phase and then were sedimented, washed once with 0.9% NaCl, and suspended in 0.5 ml of 0.9% NaCl. A 0.1-ml sample of the concentrated cells was then spread onto minimal salts-glucose plates containing thiamine (1.0 μg/ml), casein hydrolysate (0.1%), and DAP (50 μg/ml), and a crystal of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was placed on each plate. Alternatively, 10 ml of nutrient broth containing this mutagen (25 μg/ml) was inoculated with a single colony of a purine auxotroph. The cells were grown for 18 h and then were sedimented, washed once with 0.9% NaCl, and suspended in 0.5 ml of 0.9% NaCl. A 0.1-ml sample of the concentrated cells was then spread onto the same type of selective plates. After 2 to 3 days of incubation at 37°C, colonies able to utilize DAP were picked and purified on plates containing medium identical to that on which they had been selected.

RESULTS

DAP as a growth factor for purine auxotrophs. Figure 1 compares the growth of several representative purine auxotrophs in minimal medium supplemented with DAP. The *guaB5* strain can synthesize AMP de novo and only requires an exogenous source of guanine derivatives; this strain grew rapidly, implying that DAP can be efficiently converted to guanine derivatives. Conversely, strain A155 can synthesize GMP de novo and only requires an exogenous source of adenine derivatives; this strain did not grow at all, suggesting that DAP cannot be directly converted to an adenine derivative. Strain D343 is blocked in the de novo synthesis of IMP and requires an exogenous purine that can be converted to both adenine and guanine derivatives; this strain grew very slowly, suggesting that DAP cannot efficiently supply both the adenine and guanine requirements of a de novo pathway auxotroph.

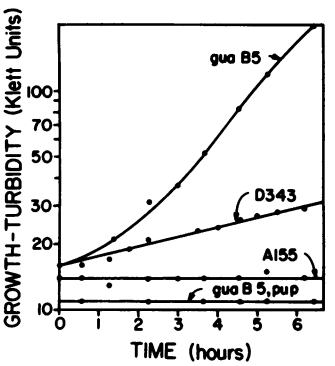

FIG. 1. *Growth curves of representative purine auxotrophs growing in minimal medium supplemented with DAP.*

An entire series of purine de novo pathway auxotrophs was tested for the ability to utilize DAP as a sole exogenous purine source (Table 2). These strains (with the exception of the *purB* strain, which is also blocked in the conversion of IMP to AMP) can use any single naturally occurring purine (e.g., adenine, guanine, hypoxanthine, xanthine, and their derivatives) to supply their requirement for adenine and guanine nucleotides. However, with the exception of the *purF* mutant (which grows well on DAP and will be discussed later), none of the representative auxotrophs can efficiently utilize DAP for this dual purpose. Table 2 also shows that it is possible to facilitate DAP utilization by addition of the glutamine analog DON to the growth medium. The probable enzymatic basis for this effect is that the presence of DON results in elevated levels of GMP reductase in *S. typhimurium* (13), thus facilitating DAP utilization by increasing the rate of conversion of GMP to IMP (see Fig. 2). Exceptions are *purA* and *purB* strains, which are blocked in the conversion of IMP to AMP; either of these mutations prohibits DAP utilization in the presence or absence of DON.

The results of these experiments are consistent with the hypothesis that DAP can be directly converted to a guanine derivative, which

TABLE 2. *Ability of purine de novo pathway auxotrophs to utilize DAP in the presence and absence of DON*

Purine de novo biosynthetic pathway[a]	Representative mutant[b]	Percent of control growth[c]	
		DAP	DAP + DON
PRPP			
↓	*purF183*	67	81
PRA			
↓	*purD312*	15	67
GAR			
↓	—[d]	—	—
F-GAR			
↓	*purG310*	15	73
F-GAM			
↓	*purI314*	<10	86
AIR			
↓	*purE66*	<10	55
C-AIR			
↓	*purC7*	<10	45
S-AICAR			
↓	*purB12*	<10	<10
AICAR			
↓	*purH190*	<10	33
F-AICAR			
↓	*purJ345*	<10	22
IMP			

[a] Abbreviations: PRPP, α-5-phosphoribosyl 1-pyrophosphate; PRA, 5-phosphoribosylamine; GAR, glycinamide ribonucleotide; F-GAR, α-*N*-formylglycinamide ribonucleotide; F-GAM, α-*N*-formylglycinamidine ribonucleotide; AIR, 5-aminoimidazole ribonucleotide; C-AIR, 5-aminoimidazole 4-carboxylic acid ribonucleotide; S-AICAR, 5-aminoimidazole 4-*N*-succinocarboxamide ribonucleotide; AICAR, 5-aminoimidazole 4-carboxamide ribonucleotide; F-AICAR, 5-formamidoimidazole 4-carboxamide ribonucleotide.

[b] Each mutant is deficient in the enzyme catalyzing the reaction immediately to its left in the diagram.

[c] Control cultures consisted of cells grown in minimal medium supplemented with hypoxanthine, except for the *purB* strain, for which minimal medium was supplemented with adenine. DON was added to a concentration of 5 μg/ml where indicated. Test cultures were grown in minimal media containing DAP.

[d] No gene or corresponding mutant has ever been identified for the phosphoribosylglycinamide formyltransferase reaction in *Salmonella* (36).

can then enter the purine salvage and interconversion pathways and be transformed into adenine derivatives.

Pathway for the utilization of DAP. Based on these growth studies and the work cited above, we postulated a probable route for DAP metabolism, via the purine salvage enzymes, whereby it could yield guanine derivatives (Fig. 2). DAP could first be condensed with ribose 1-phosphate, in a reversible reaction catalyzed by purine nucleoside phosphorylase, to form DAP ribonucleoside. This could then be deaminated at the 6-carbon position by adenosine deaminase to yield guanosine, a naturally occurring purine nucleoside which is easily metabolized to guanine and adenine nucleotides via the purine salvage and interconversion pathways.

To verify this pathway experimentally, we first tested whether a *guaB deoD* strain, which only requires an exogenous source of guanine nucleotides and which totally lacks purine nucleoside phosphorylase activity, can utilize DAP. Its parent strain (*guaB5*) readily utilizes DAP. However, the *deoD* mutant did not grow at all when DAP was the sole source of purines (Fig. 1). From this we infer that the enzyme, purine nucleoside phosphorylase, is required for the utilization of DAP as a source of guanine compounds.

To verify whether adenosine deaminase is required for DAP utilization we took advantage of another unique property of DON. In addition to causing increased GMP reductase levels and facilitating DAP utilization, DON can create a requirement for exogenous purines in *S. typhimurium* by inhibiting the two glutamine-dependent amidotransferase reactions required for de novo synthesis of IMP (7, 16). The latter

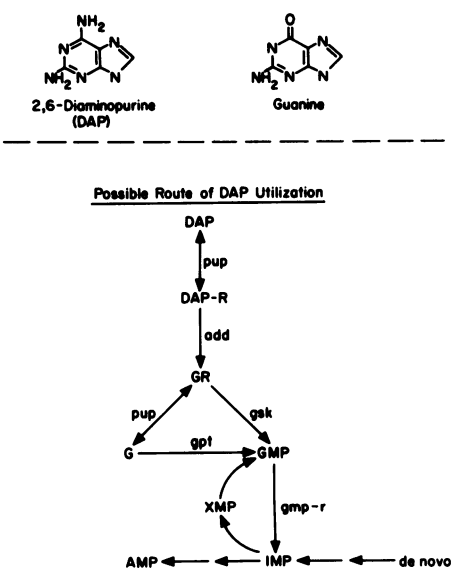

FIG. 2. *Postulated route of DAP utilization via purine salvage and interconversion enzymes. Abbreviations: G, guanine; DAP-R, DAP ribonucleoside; GR, guanosine; pup, purine nucleoside phosphorylase; add, adenosine deaminase; gsk, guanosine kinase; gpt, guanine phosphoribosyltransferase; gmp-r, GMP reductase.*

property is important because the strains used to test the requirement of adenosine deaminase for DAP utilization were purine prototrophs. Figure 3 compares the growth of strain LT2 and a mutant which completely lacks adenosine deaminase activity in minimal media containing DAP and DON (40 μg/ml). Only the strain with adenosine deaminase activity could grow on DAP, implying that this enzyme has an essential role in DAP utilization.

Isolation and characterization of mutants able to use DAP. Having shown that the probable route of DAP utilization is through its conversion to DAP ribonucleoside, followed by deamination to guanosine, we proceeded to isolate strains with genetic lesions that would allow efficient utilization of DAP by purine auxotrophs. We thought this might be a useful approach for isolating mutants defective in the regulation of the enzymes catalyzing the necessary reactions (e.g., purine nucleoside phosphorylase, adenosine deaminase, GMP reductase). It is known that guanosine can easily satisfy the requirement for both adenine and guanine nucleotides in strains blocked in the de novo synthesis of IMP. Guanosine is converted to GMP in one or two steps, induces GMP reductase (4), and thereby speeds the conversion of guanine nucleotides to adenine nucleotides. However, as indicated above, DAP, which is converted to guanosine in two steps, can only efficiently supply the guanine but not the adenine nucleotide pool. This suggests that the rate of conversion of DAP to AMP via GMP is the growth rate-limiting process. Thus, any mutation which results in elevated levels of any of the enzymes required for the conversion of DAP to AMP via GMP might allow efficient utilization of DAP. Toward this end, several purine auxotrophs blocked in the synthesis of IMP were mutagenized, and selection was made for mutants which could efficiently grow with DAP as the sole purine source, as described in Materials and Methods. Several such mutants were obtained, and the growth properties of representative strains are shown in Fig. 4.

Figure 4 compares the growth rates of a *purG* derivative and its parent strain in minimal media containing hypoxanthine or DAP. The parent strain, G310, grew well on hypoxanthine, with a generation time of 29 min, but hardly at all on DAP. The DAP-utilizing mutant, GP70, likewise grew well on hypoxanthine (generation time, 39 min), but, unlike its parent, could efficiently utilize DAP (generation time, 82 min). Dose-response analyses showed that half-maximal growth was obtained with 20 μg of either hypoxanthine or DAP per ml with the DAP-utilizing mutant. The parent showed the same response to hypoxanthine but did not grow significantly in DAP up to the highest concentration tested (100 μg/ml).

A total of seven representative DAP-utilizing mutants were assayed in comparison with their parents for the following enzymes: purine nucleoside phosphorylase, thymidine phosphorylase, adenosine deaminase, and GMP reductase. Three (e.g., strain GP73) showed no significant differences in any of these enzymes and remain unclassified. Four of the remaining strains selected on the basis of DAP utilization were found to have fully constitutive levels of purine nucleoside phosphorylase and thymidine phosphorylase (Table 3) and hence were phenotypically classified as *deoR* mutants. Fully constitutive levels of these two enzymes, in the absence of

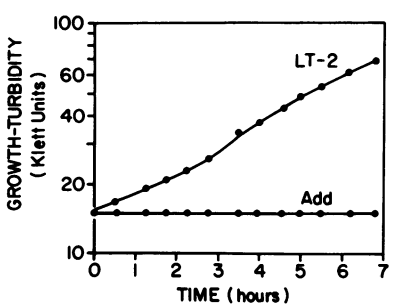

FIG. 3. *Growth curves of the wild-type strain (LT-2) and a derivative completely lacking adenosine deaminase activity (Add) in minimal media supplemented with DON (40 μg/ml) and DAP. Neither strain grew in control medium lacking DAP, and both strains grew rapidly in control medium containing DON (40 μg/ml) and hypoxanthine (data not shown).*

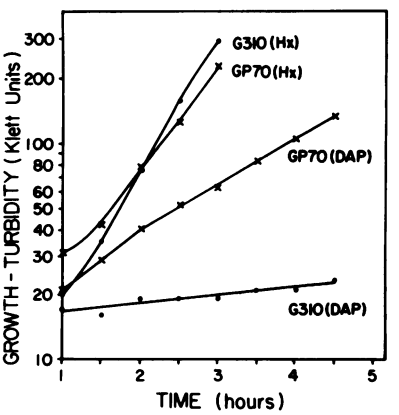

FIG. 4. *Growth curves of a purine auxotroph (G310) and a DAP-utilizing derivative (GP70) in minimal medium supplemented with hypoxanthine (Hx) or DAP.*

TABLE 3. *deo regulon enzyme levels*[a] *in DAP-utilizing purine auxotrophs*

Strain	Relevant genotype	Purine nucleoside phosphorylase	Thymidine phosphorylase
LT-2	Wild type	358	146
G310	*purG*	373	128
GP70	*purG deoR*	4,915	3,390
GP71	*purG deoR*	4,056	5,700
D343	*purHD*	346	133
GP72	*purHD deoR*	3,544	6,000
SL751	*purC purI*	506	180
GP88	*purC purI deoR*	3,167	10,313
GP73	*purC purI DPU*[b]	397	186

[a] Enzyme levels were determined as described in the text and are expressed as specific activities. The strains were grown in minimal medium supplemented with hypoxanthine (50 μg/ml), thiamine, and the appropriate amino acids, as required.

[b] DPU, Unclassified DAP-utilizing mutant.

inducers, was taken as presumptive evidence of a *deoR* lesion (6). The *deo* genes comprise a regulon composed of at least two tandem operons, *deoC-deoA* and *deoB-deoD*, with thymidine phosphorylase (*deoA*) coded in the former and purine nucleoside phosphorylase (*deoD*) in the latter (34). A mutation in the regulatory gene *deoR* is the only known defect that can yield this enzyme pattern. Mutations in the *cytR* regulatory gene result in only partially constitutive levels of the *deo* enzymes (30), and an operator-constitutive mutation would result in constitutivity of either thymidine phosphorylase or purine nucleoside phosphorylase, not both. These strains had normal levels of cytidine deaminase, further ruling out *cytR* lesions, along with normal levels of adenosine deaminase and GMP reductase (data not shown).

DISCUSSION

In this investigation we examined the pathway for the utilization of DAP as an exogenous purine source in *S. typhimurium*. Purine nucleoside phosphorylase and adenosine deaminase seem to be absolutely required for DAP utilization, indicating that the most probable route of metabolism is via the conversion of DAP to its nucleoside form, followed by deamination at the 6-carbon position to yield guanosine.

When purine auxotrophs blocked in the de novo synthesis of IMP were mutagenized and selected for the ability to use DAP as the sole exogenous purine source, the derivative strains fell into several categories. One category consisted of strains with no discernible alteration in the levels of the candidate enzymes, and their mechanism for utilizing DAP remains unknown. Strains with constitutive levels of purine nucleoside phosphorylase (Table 3) constitute another category. This finding again supports the important role of purine nucleoside phosphorylase in the utilization of DAP. Although not directly tested in this investigation, it seems likely that DAP is a poor substrate for purine nucleoside phosphorylase, and hence fully constitutive levels of the enzyme are required for efficient conversion of DAP to its riboside form. A similar phenomenon is observed when purine auxotrophs unable to synthesize IMP de novo are mutagenized and selected for the ability to utilize 9-β-D-arabinofuranosyladenine as the sole exogenous purine source. Strains constitutive for purine nucleoside phosphorylase are consistently isolated (Garber and Gots, manuscript in preparation).

All (15/15) of the *purF* strains in our collection are able to grow on DAP (one example is given in Table 2). Purine auxotrophs which only possess *purF* lesions may possibly owe their ability to utilize DAP to the existence in *Salmonella* of an alternative enzyme which synthesizes phosphoribosylamine from ribose 5-phosphate and ammonia (27), as well as a nonenzymatic pathway for this reaction (29). These reactions might bypass the defective α-5-phosphoribosyl 1-pyrophosphate amidotransferase reaction in the *purF* strains (see Fig. 2) and allow enough de novo synthesis of AMP so that DAP would only have to supply the guanine nucleotide requirement.

One class of mutants that might be expected to utilize DAP are strains with constitutive levels of adenosine deaminase. No such strains were isolated in the present study; this could be due to the fact that adenoside deaminase is reported to be noninducible in *S. typhimurium* (31). Another class of mutants that might be expected to use DAP are strains with elevated levels of GMP reductase. Although no such strains were isolated, this mechanism is probably a valid one, because of the findings that (i) DON allows most purine auxotrophs blocked in the synthesis of IMP to use DAP as sole exogenous purine source (Table 2), and (ii) DON causes a dramatic increase in GMP reductase levels in *S. typhimurium* (13).

The role of guanine phosphoribosyltransferase in DAP utilization is more difficult to assess. The reason for this is that DAP-riboside is deaminated to guanosine, which can either be phosphorylated directly to GMP via guanosine kinase, or be phosphorolytically cleaved to guanine and ribose 1-phosphate by purine nucleoside phosphorylase. Guanine can then be converted to GMP via guanine phosphoribosyl-

transferase (*gpt*). In strains able to utilize DAP by virtue of constitutive levels of purine nucleoside phosphorylase, much of the guanosine formed from DAP might undergo the phosphorolytic reaction, thus giving guanine phosphoribosyltransferase an important role in reclaiming guanine. In addition, the two-step pathway allows the catalytic participation of ribose 1-phosphate. This compound is used when DAP is converted to its riboside, and regenerated when guanosine is converted to guanine. This is biologically more efficient than metabolism via the guanosine kinase reaction, since the latter creates a net deficit of 1 mol of ribose 1-phosphate per mol of guanosine utilized.

In all other DAP-utilizing strains, *gpt* might be expected to play a lesser but still important role. The role of *gpt* was tested by comparing the growth of two pairs of strains: GP66 (*gpt*) and GP66/F'128 (*gpt*$^+$) in minimal medium containing DON (5 μg/ml) and DAP, along with GP67 (*gpt*) and GP67/F'128 (*gpt*$^+$) in minimal medium just containing DAP. Both *gpt* strains grew only to a limited degree, whereas the *gpt*$^+$ strains grew rapidly (data not shown). Limited growth of the *gpt* strains could mean that *gpt* facilitates DAP utilization, but is not absolutely required for it, as indicated in Fig. 2. Growth under these conditions could also be aided by the hypoxanthine-specific phosphoribosyltransferase (*hpt*) in *Salmonella*, which is known to have slight activity with guanine as a substrate (8).

Previous studies (14, 21, 22) on the inhibition of growth by DAP employed high concentrations of the analog (e.g., 500 μg/ml); however, it has been shown that lower concentrations (e.g., 40 μg/ml) will not inhibit the growth of *S. typhimurium* LT2 (21). Other studies in this laboratory, comparing DON-stimulated DAP utilization by a strain totally lacking adenine phosphoribosyltransferase (*apt*) activity (and hence unable to convert DAP to its inhibitory nucleotide form) with that of its *apt*$^+$ parent, revealed no significant difference in their ability to efficiently use DAP (data not shown). Thus, growth inhibition by DAP does not seem to play an important role at the concentration (50 μg/ml) employed in this investigation.

In summary, DAP, although it can easily supply the guanine nucleotide requirements of a strain blocked in the conversion of IMP to GMP, cannot supply both the guanine and adenine nucleotide requirements of a strain unable to synthesize IMP de novo. The most likely reason for this phenomenon is that there is normally a limitation in the rate of conversion of DAP to GMP and then to AMP. This limitation may be overcome by mutations (e.g., *deoR*) or conditions (e.g., the administration of DON) resulting in elevated levels of the enzymes needed for this conversion. Other mechanisms for DAP utilization exist (e.g., in *purF* mutants), but these remain unclear at present.

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