Involvement of the Oxidative Pentose Phosphate Pathway in Thiamine Biosynthesis in *Salmonella typhimurium*

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purF mutants of *Salmonella typhimurium* are known to require a source of both purine and thiamine; however, exogenous pantothenate may be substituted for the thiamine requirement. We show here that the effect of pantothenate is prevented by blocks in the oxidative pentose phosphate pathway, *gnd* (encoding gluconate 6-phosphate [6-P] dehydrogenase) or *zwf* (encoding glucose 6-P dehydrogenase). We further show that the defects caused by these mutations can be overcome by increasing ribose 5-P, suggesting that ribose 5-P may play a role in the ability of pantothenate to substitute for thiamine.

Aminoimidazole ribotide (AIR) is a precursor both of purines and of thiamine pyrophosphate (Fig. 1). The well-known purine biosynthetic pathway includes the enzymes that synthesize AIR, and mutants blocked in steps prior to AIR, such as *purF*, whose product makes phosphoribosylamine (PRA), are auxotrophic for both purines and thiamine. However, there appear to be other routes to AIR which can suffice to meet the cell's (low) thiamine requirement, and genetic analyses have implicated anaerobiosis, carbon metabolism, and pantothenate metabolism in these alternative routes (3, 5, 16).

The alternative pyrimidine biosynthetic (APB) pathway appears to lead to AIR by reactions as yet unknown. Mutants defective in the APB pathway have been isolated on the basis of their requiring thiamine anaerobically, i.e., *purF purG apbA* (4).

The role of pantothenate in thiamine synthesis has been unclear. One speculation (3) placed it as a precursor in the APB pathway, but labeling studies have disproved this hypothesis (8). Other experiments seemed to implicate pantothenate in an alternative route to PRA (5). We have now constructed a *purF apbA* double mutant (DM587) and observe that this strain, like a *purF* mutant, grows with adenine and either thiamine or pantothenate. This result suggests that pantothenate is indeed involved in a pathway separate from APB, depicted in Fig. 1 as the pantothenate-dependent pyrimidine (PDP) pathway. The PDP pathway has been shown to only bypass the PurF enzyme, because the thiamine requirement of purine mutants blocked subsequent to this step cannot be met by pantothenate (5).

Isolation of Pdp mutants. We proceeded to analyze the PDP pathway by obtaining derivatives of *purF apbA* strains in which pantothenate no longer substituted for the thiamine requirement. A P22 lysate grown on a pool of >60,000 cells containing either random MudJ or Tn*10*d(Tc) insertions was used to transduce DM587 [*purF2085 apbA7*::Tn*10*d(Tc)] or DM384 (*purF2085 apbA1*::MudJ) to the appropriate antibiotic resistance. The media, chemicals, and genetic techniques used in this work have been described previously (4). Screening of >50,000 antibiotic-resistant transductants revealed 18 mutants with the desired phenotype. Such mutants might be blocked in either the PDP pathway or after PRA (e.g., *purD, -G, -I,* or *thi*

genes). The latter class of mutants would be expected to cause a purine or thiamine auxotrophy when transduced into a wild-type (*purF*[+]) background, and by such a test, 6 of the 18 mutants proved to be purine auxotrophs. The remaining 12 mutants had mutations that were silent in a wild-type background and thus were designated as putative *pdp* mutants. Four of these 12 were somewhat "leaky" and were not further characterized.

Identification of Pdp mutants as defective in Gnd and Zwf. The eight mutations that resulted in a clear Pdp phenotype but caused no auxotrophy in a wild-type background were identified as lesions that blocked the oxidative pentose phosphate pathway: three *zwf* and five *gnd* mutations (Fig. 2). The *gnd* mutations were recognized by a chance observation of their transductional linkage to the histidine operon. When a strain containing one of our *gnd* mutations was used to transduce a *his-640* strain with a deletion extending from the *hisEI* locus through the *gnd* gene, 100 of 100 antibiotic-resistant transductants were His[+]. A plasmid carrying the *gnd* gene from *Escherichia coli* (pMN6) (13, 14) restored the Pdp[+] phenotype in our *gnd* mutants, while two *gnd* mutant plasmids (pMN7 and pMN8) (14) did not. In addition, the introduction of a *pgi* mutation into one of our *gnd* mutants resulted in a strain unable to grow on glucose (as known for *pgi gnd* double mutants [11]). The *zwf* mutation was identified analogously: a double mutant with *pgi* caused glucose negativity, and a plasmid carrying the *E. coli zwf* gene (pDR17) (18) restored the Pdp[+] phenotype. In addition, our *zwf* mutation mapped to the region of the chromosome in which *zwf* is located in *E. coli* according to the method of Liu and Sanderson (12).

Comparison of the Pdp phenotype aerobically versus anaerobically. Table 1 shows the specific growth rates in minimal glucose medium with the indicated supplements for the parental *purF apbA* strain (DM587) and the *gnd* mutant derivative strain (DM589). Specific growth rates were calculated as described previously (3). As expected from the screening, pantothenate was unable to substitute for the thiamine requirement in the *gnd* mutant when standard aerobic shaking cultures were used. Interestingly, in standing cultures the results were more complicated: the thiamine or pantothenate requirement was only partial in the *purF apbA* parental strain, although adenine was still required, and pantothenate still had some effect in the *gnd* mutant. Standing cultures are considerably more anaerobic than shaking cultures, and it is known that the oxidative branch of the pentose phosphate pathway is impaired anaerobically (6, 7). The fact that a *gnd* mutation results in a weaker

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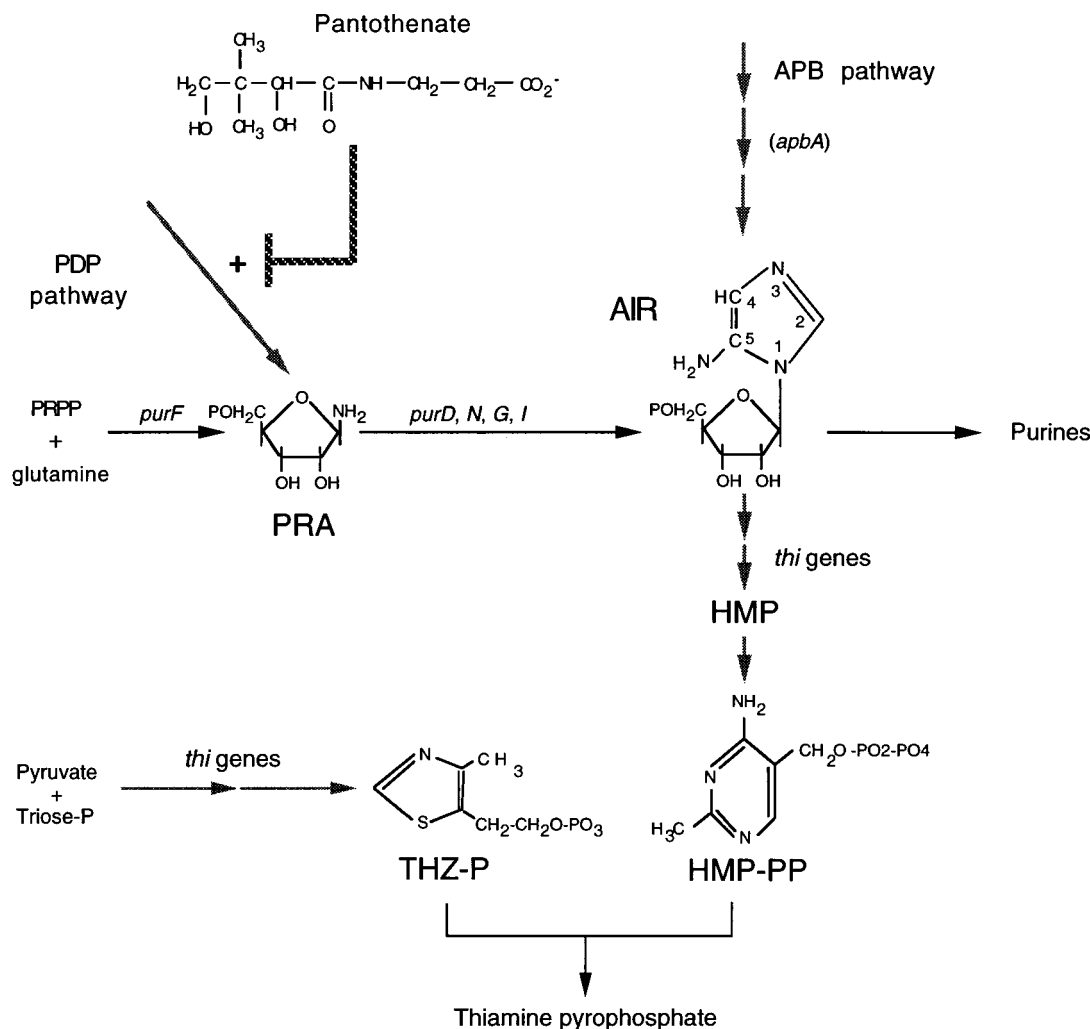
FIG. 1. Biosynthetic pathways involved in thiamine synthesis. Genes whose products are required for selected reactions are indicated beside the relevant arrow. The positive role of pantothenate in the PDP pathway is indicated (5). The products of the 4-amino-5-hydroxymethyl-2-methyl-pyrimidine (HMP) and thiazole (THZ) pathways, HMP-PP and THZ-P, respectively, are joined, and a subsequent phosphorylation step forms thiamine pyrophosphate, the active form of the coenzyme. The designation of AIR as the product of the APB pathway is based on genetic data (16).

Pdp phenotype under conditions in which the Gnd enzyme is used less is in agreement with the Gnd requirement for the PDP pathway being related to its known normal function (e.g., formation of ribose 5-phosphate [5-P] for the proposed pantothenate-dependent route of PRA synthesis).

Conditions that increase ribose 5-P levels restore PDP pathway function in *gnd* and *zwf* mutants. According to the speculation presented above, other sources of ribose 5-P might suppress the Pdp phenotype caused by *gnd* or *zwf* mutations. Indeed, when ribose was used as the sole carbon source (Fig. 2) with the triple mutants DM923 [*purF2085 apbA7*::Tn*10*d(Tc) *zwf25*::MudJ] and DM589 [*purF2085 apbA1*::MudJ *gnd175*::Tn*10*d(Tc)], their growth with the adenine-plus-pantothenate supplement was indistinguishable from that of the *purF apbA* parent. Consistent with this result, when gluconate was used as the sole carbon source, bypassing the need for Zwf (Fig. 2), the *purF apbA zwf* mutant (DM923) grew with adenine plus pantothenate while the *purF apbA gnd* mutant (DM589) did not. In addition, we found that either ribose 5-P or ribose could substitute for the thiamine requirement of a *purF apbA gnd* mutant (DM589) on a minimal plate containing adenine and pan-

tothenate (Fig. 3), provided that appropriate conditions were employed (induction by glucose 6-P for pentose P uptake [20], or overcoming catabolite repression for use of ribose [2]). Similar results were obtained with a *purF apbA zwf* mutant (DM923). It is important to note that all of the conditions described above which would increase ribose 5-P levels to suppress either *gnd* or *zwf* mutations still required pantothenate. These data indicate that pantothenate does not substitute for thiamine synthesis by increasing cellular ribose 5-P.

We also employed the well-characterized *prs-100* mutation (15, 17), which decreases phosphoribosylpyrophosphate (PRPP) synthetase activity and cellular PRPP pools to ca. 20% of wild-type levels. The mutant enzyme has an increased K_m for both ribose 5-P and ATP (17) and might be expected to cause an increase in ribose 5-P levels, since PRPP synthetase consumes a significant amount of cellular ribose 5-P. The *prs-100* mutation was introduced into DM589 by its linkage to *hemA* (19), resulting in the quadruple mutant DM2013 [*purF2085 apbA1*::MudJ *gnd175*::Tn*10*d(Tc) *prs-100*]. This mutant had the expected phenotype at 30°C, i.e., no growth with adenine
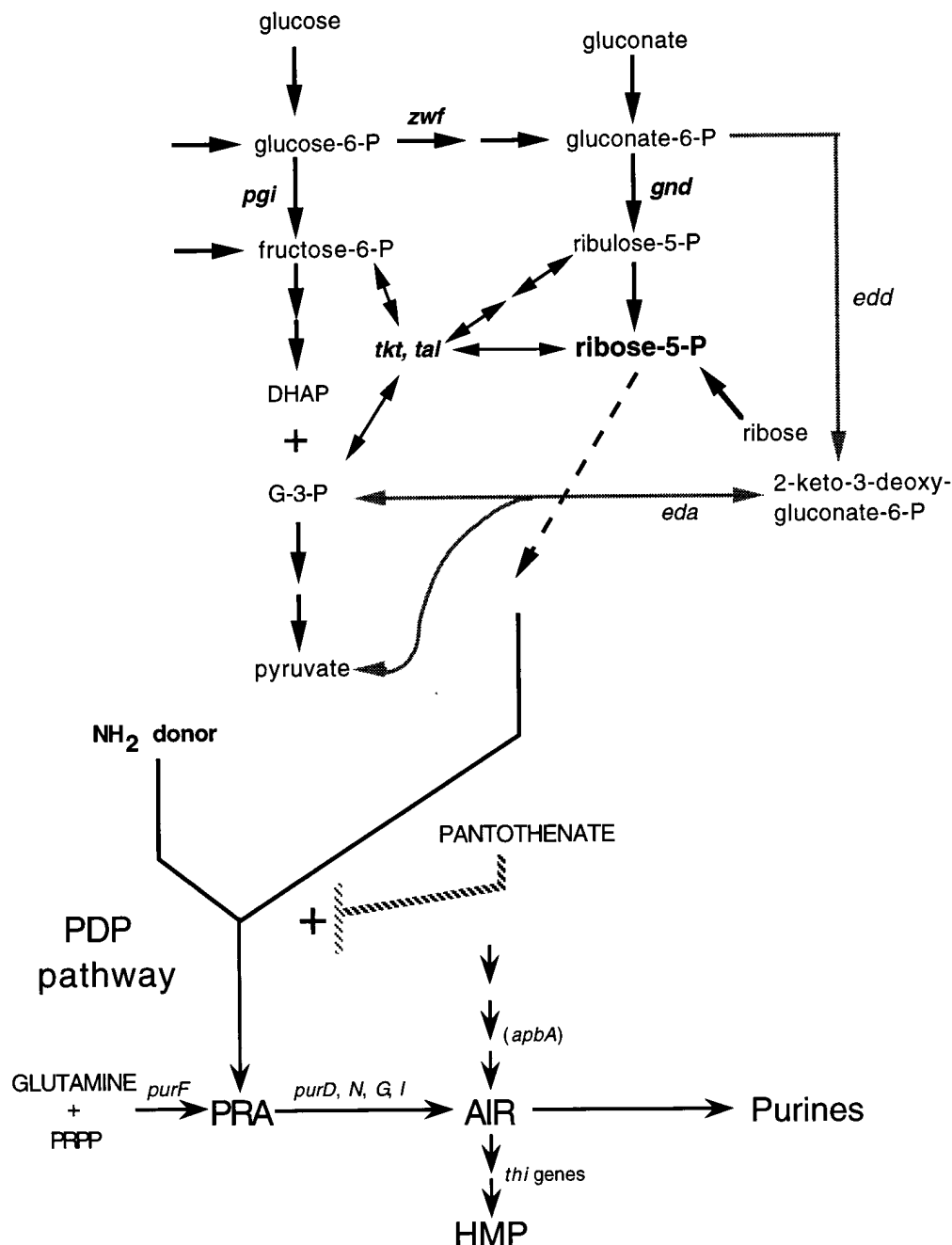
FIG. 2. Model of the connection between the pentose phosphate pathway and the PDP pathway. Glycolysis, the pentose phosphate pathway, and the Entner-Doudoroff pathway are schematically represented in the top portion of the figure. Dihydroxyacetone-phosphate (DHAP) and glyceraldehyde 3-P (G-3-P) can be interconverted by triosephosphate isomerase. Two arrows are used to represent the five steps which convert glyceraldehyde 3-P to pyruvate. The proposed involvement of the pentose phosphate pathway in the PDP pathway is indicated by a dashed line. Proposed substrates for PRA formation are ribose 5-P and an unspecified amine donor. The genes whose products are required for selected reactions are indicated by the relevant arrow. HMP, 4-amino-5-hydroxymethyl-2-methyl-pyrimidine.

TABLE 1. Effect of a *gnd* mutation on the PDP pathway in aerobic versus anaerobic growth conditions

Strain	Genotype	Specific growth rate (μ) (doublings h^{-1}) in[a]:					
		Shaking culture supplemented with:			Standing culture supplemented with:		
		ade	ade + pan	ade + B_1	ade	ade + pan	ade + B_1
DM587	*purF2085 apbA7*::Tn*10*d(Tc)	0.03	0.42	0.50	0.18	0.33	0.28
DM589	*purF2085 apbA1*::MudJ *gnd175*::Tn*10*d(Tc)	0.03	0.01	0.53	0.09	0.18	0.25

[a] Growth curves were determined as described in reference 4. Standing conditions result in low oxygen levels in the medium (3); overlaying cultures with mineral oil did not result in any significant changes. Data presented are representative of five independent experiments. Abbreviations: ade, adenine; pan, pantothenate; B_1, thiamine.
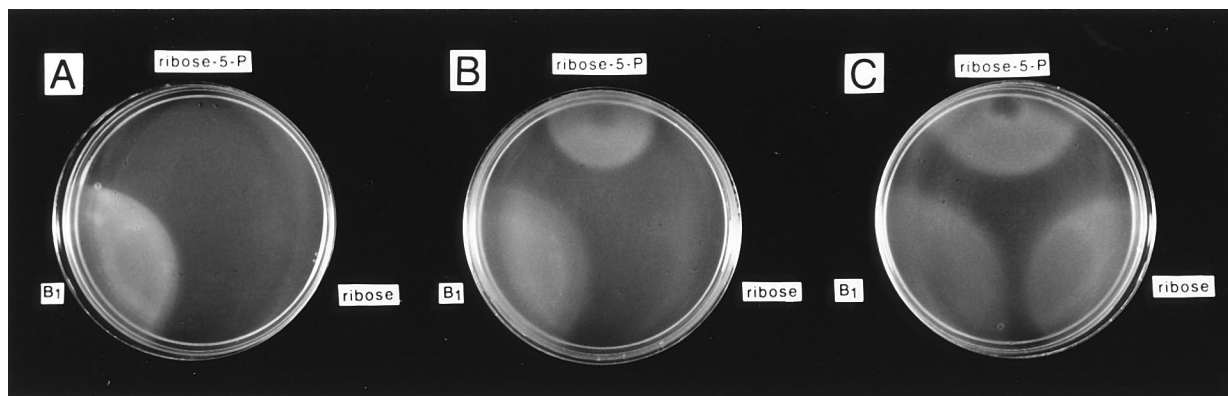
FIG. 3. Correction of the defect in thiamine synthesis caused by a *gnd* mutation by ribose and ribose 5-P. Soft agar overlays of DM589 (*purF apbA gnd*) were performed as described previously (5). Cells were plated on minimal medium supplemented as follows: A, glucose and adenine; B, glucose, adenine, and pantothenate; C, succinate, adenine, and pantothenate. A 10-μl sample containing 20 mmol of either ribose or ribose 5-P and a 1-μl sample containing 10 nmol of thiamine (B₁) were added where indicated. Glucose 6-P was added at a concentration of 0.4 mM to induce the sugar phosphate transport system. In no case did glucose 6-P alone have any effect.

and pantothenate but weak growth with adenine and pantothenate at 37°C. This growth depended on pantothenate, as if *prs-100* was indeed affecting ribose 5-P rather than causing a different mechanism of relief of the thiamine requirement. This result strongly supports the hypothesis that blocks in the oxidative pentose phosphate pathway cause defects in the PDP pathway because of a reduction of ribose 5-P pools. As expected, the *prs*⁺ control strain [*purF2085 apbA1*::MudJ *gnd175*::Tn*10*d(Tc)] did not grow with adenine and pantothenate at any temperature. In addition, as expected, strains containing *prs-100* did not grow at 42°C even with a thiamine supplement. Considering that relative anaerobiosis might analogously impair this reaction by altering ATP/ADP ratios, which regulate PRPP synthetase (9), we tested strain DM589 [*purF2085 apbA1*::MudJ *gnd175*::Tn*10*d(Tc)] on glucose medium containing adenine and pantothenate and KCN (100 μM), but growth was not restored. This result indicates that the effects observed with standing cultures were not due to altered PRPP synthetase activity.

Taken together, these data strongly support involvement of ribose 5-P in the PDP pathway. A model incorporating ribose 5-P as a precursor to PRA in a pantothenate-dependent reaction is shown in Fig. 2. A functioning oxidative pentose phosphate pathway would be required to provide sufficient ribose 5-P for this reaction to occur. This model predicts the ribose 5-P pool to be lower in *zwf* and *gnd* mutants. In preliminary results, unfortunately, assays of ribose 5-P (1, 10) have not revealed clear differences in the blocked mutants. However, the differences are expected to be small, considering that *zwf* and *gnd* mutants are not starved for other ribose 5-P-derived compounds.

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