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Metabolic Relationships between Pyridoxine (Vitamin B_6) and Serine Biosynthesis in *Escherichia coli* K-12

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We propose a pathway leading from erythrose-4-phosphate and glutamate to nitrogen 1 and carbons 5, 5′, and 6 of the pyridoxine ring. This pathway, which parallels the phosphorylated pathway of serine biosynthesis, is predicated on the homology between PdxB and SerA, the structural similarity between serine and 4-hydroxythreonine, and the possible involvement of SerC in pyridoxine biosynthesis. Several predictions of this hypothetical scheme were tested. Consistent with the proposed pathway, supplement inhibition patterns strongly suggest that SerA enzyme acts in an alternate pathway of pyridoxine biosynthesis in *pdxB* mutants. Direct enzyme assays detected erythrose-4-phosphate dehydrogenase activity in crude extracts, which again supports the proposed pathway. Chromosomal insertions in *serC* caused a requirement for pyridoxine, serine, and aromatic compounds, which directly verified that SerC functions in the pyridoxine biosynthetic pathway. Complementation analysis showed that *pdxF* and *pdxC* mutations reported previously are most likely alleles of *serC*. Growth of *serC* chromosomal insertion mutants on glycolaldehyde was found to occur without acquisition of second-site mutations and confirmed that *pdxB* and *serC*, but not *pdxA*, function in the same branch of the pyridoxine pathway. In addition, *serC*::mini-Mu d insertions revealed that the complex *serC-aroA* operon lacks internal promoters, that the amino terminus of SerC is not strictly essential for activity, and that antisense transcription occurs in the *serC-aroA* operon. Growth responses of *pdxA*, *pdxB*, and *serC* mutants to β-hydroxypyruvate, D-alanine, and glycolate could also be reconciled with the proposed pathway. Finally, the proposed scheme is consistent with previous isotope labeling data and accounts for several other observations about pyridoxine biosynthesis. Together, these physiological and biochemical analyses support the proposed pathway and an evolutionary scenario in which this branch of the pyridoxine pathway evolved from the serine pathway by gene recruitment.

Pyridoxine (vitamin B_6) is the pyridine ring-containing precursor of essential coenzyme pyridoxal phosphate, which is utilized by enzymes in all phases of amino acid metabolism (7). Pyridoxine is synthesized by numerous bacteria, fungi, and higher plants, whereas the steps that convert pyridoxine into pyridoxal phosphate appear to be found in all organisms (52). Because of its importance to amino group metabolism, pyridoxine and pyridoxal phosphate biochemistry have been studied intensively for over 30 years (for reviews, see references 18, 19, 27, and 29), yet the pyridoxine biosynthetic pathway remains largely unknown.

Combined genetic, physiological, and biochemical analyses using *Escherichia coli* B and K-12 strains suggest two likely precursors of pyridoxine. Consideration of nutritional requirements and isotope labeling patterns led Dempsey to propose that 4-hydroxythreonine undergoes a decarboxylation and then supplies nitrogen 1 and carbons 5, 5′, and 6 to the pyridoxine ring (Fig. 1) (19). Recent nuclear magnetic resonance analyses by Hill and co-workers implicate D-1-deoxyxylulose or a 4-oxo derivative as the donor of remaining carbons 2, 2′, 3, 4, and 4′ to the pyridoxine ring (28). However, the steps leading to these putative intermediates have been neither delineated nor related to functions of the mapped pyridoxine (*pdx*) biosynthetic genes, which fall into at least five linkage groups and are members of complex, multifunctional operons in *E. coli* K-12 (Lam and Winkler, unpublished results) (1, 22, 40).

Recently, we reported that the *pdxB* and *serA* gene products of *E. coli* K-12 are evolutionarily related (42). The *serA* gene encodes 3-phosphoglycerate dehydrogenase, which catalyzes the first step of the major phosphorylated pathway of serine biosynthesis (Fig. 1) (47). This analysis strongly suggests that the *pdxB* gene product is a 2-hydroxyacid dehydrogenase and that the branch of pyridoxine biosynthesis mediated by *pdxB* evolved from the phosphorylated pathway of serine biosynthesis (42). Further, the close structural similarity between serine and 4-hydroxythreonine and the possible involvement of *serC*-encoded phosphoserine aminotransferase in pyridoxine biosynthesis (20, 30, 44) suggest the pathway depicted in Fig. 1 (42). In this scheme, the *pdxB* gene product encodes erythronate-4-phosphate dehydrogenase and the SerC aminotransferase acts at a point parallel to the phosphorylated serine biosynthetic pathway.

In this paper, we test several predictions based on the hypothetical scheme shown in Fig. 1. In particular, we manipulate alternate pathways in various *pdx* insertion mutants to gain new information about the pathways leading to pyridoxine. We also reexamine the possible roles of several putative intermediates, such as glycolaldehyde, β-hydroxypyruvate, and D-alanine, in pyridoxine biosynthesis. The results are consistent with the pathway in Fig. 1, which in turn is consistent with all previous isotope labeling data.

MATERIALS AND METHODS

Materials. Enzymes used in cloning *serC* were the following: restriction endonucleases, T4 DNA ligase, and phosphorylated *Sal*I linkers (New England BioLabs, Beverly, Mass.); T4 DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.); and bacterial alkaline phos-

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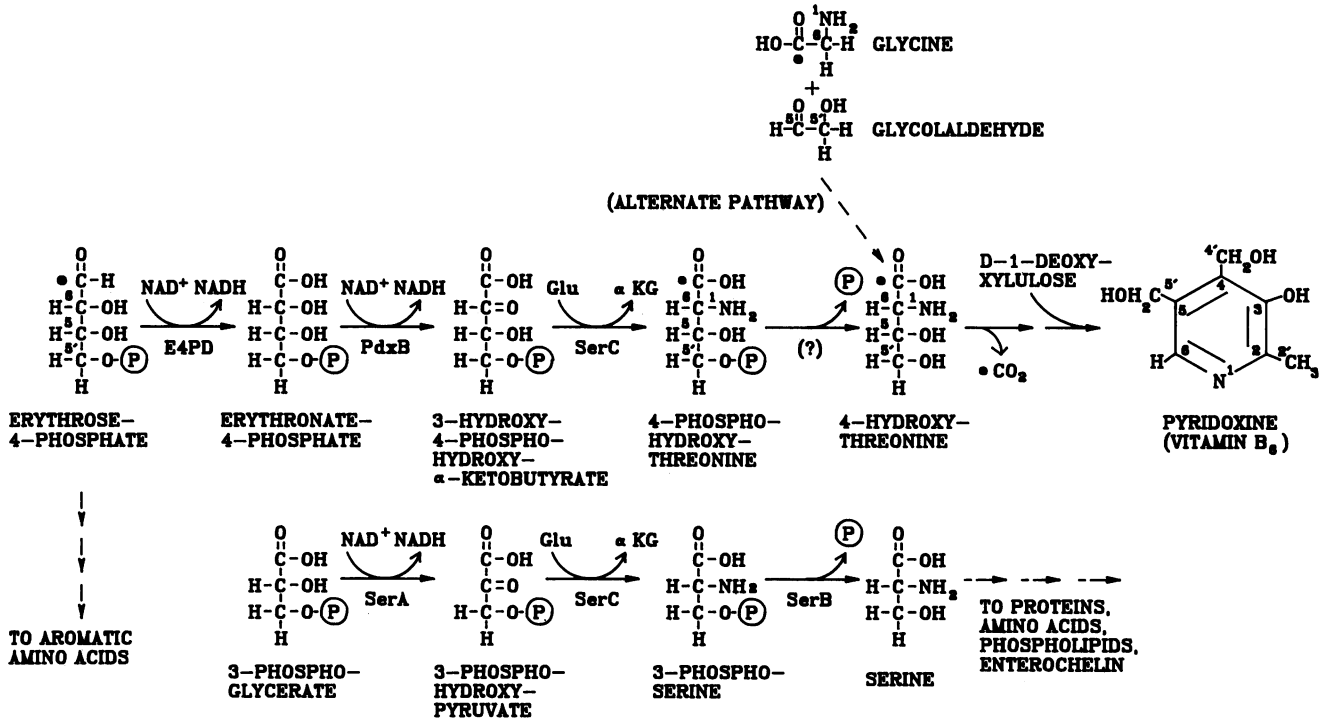

FIG. 1. Proposed pathway for the entrance of nitrogen 1 and carbons 5, 5', and 6 into the intermediate, 4-hydroxythreonine, and the pyridine ring of pyridoxine (vitamin B_6). The major phosphorylated pathway of serine biosynthesis is drawn below and in parallel to the proposed branch of the pyridoxine pathway. An alternate pathway from glycine and glycolaldehyde that can be detected in *pdxB* and *serC* mutants is also shown (see text). The numbers on the nitrogen and carbon atoms refer to final positions of the pyridoxine ring, and the dot marks the carbon of 4-hydroxythreonine lost during decarboxylation. The pathway is based on genetic, physiological, and isotope labeling data (see text), and the intermediates shown have not yet been detected chemically. Enzyme designations: E4PD, erythrose-4-phosphate dehydrogenase; PdxB, likely erythronate-4-phosphate dehydrogenase (see text); SerC, phosphoserine aminotransferase; (?), hypothetical phosphatase activity encoded by *pdxJ* or another gene (see text); SerA, D-3-phosphoglycerate dehydrogenase; SerB, 3-phosphoserine phosphatase.

phatase (Bethesda Research Laboratories, Gaithersburg, Md.). Antibiotics, biochemicals, and crystalline D-erythrose-4-phosphate were purchased from Sigma Chemical Co. (St. Louis, Mo.). Ingredients for culture media were from Difco Laboratories (Detroit, Mich.). Inorganic salts and electrophoresis-grade agarose were bought from Fisher Scientific (Fair Lawn, N.J.).

Bacterial strains, plasmids, media, and culture conditions. Bacterial strains and plasmids used in this study are listed in Table 1. Markers were moved between strains by generalized transduction using P1 *kc* or P1 *vir* bacteriophage as described previously (35). Recombinant plasmids were constructed using standard techniques (3, 41). Insertional mutagenesis of plasmids with mini-Mu dI(Km^r) and mini-Mu dII(Km^r) was performed as previously described (10). The mini-Mu dI elements used (Table 1) form stable insertions that cannot transpose further (10). Insertion positions in plasmids were determined by restriction analysis using several enzymes (3, 41) and are accurate to within about 50 bp. Insertions imparting resistance to kanamycin were crossed from linearized plasmids into the chromosome of *recBC sbc* mutant JC7623 as detailed before (2). Chromosomal insertions were moved into Δ*lac* Δ*trp* mutant VJS433 and Δ*lac* mutant NU816 to form isogenic sets of strains (Table 1).

LBC rich medium was Luria-Bertani broth supplemented with 30 μg of L-cysteine per ml. Minimal Vogel-Bonner 1XE medium containing 0.01 mM $FeSO_4$ was prepared as previously described (12). Supplements are listed in the table footnotes and figure legends. $FeSO_4$ was omitted from minimal salts medium containing the six aromatic compounds, L-Trp, L-Tyr, L-Phe, 4-aminobenzoate, 4-hydroxybenzoate, and 2,3-dihydroxybenzoate (see Table 4). For strains with complicated genotypes (Table 1), additional nutritional requirements were added at concentrations suggested by Davis et al. (12). Liquid cultures were grown with vigorous shaking in Nephelo flasks at the temperatures indicated, and their turbidities were monitored by using a Klett-Summerson colorimeter. Growth tests were performed by streaking cells onto prewarmed plates, by inoculating liquid cultures with a small amount of washed cells, or by using crystal spot tests (1).

Pseudorevertants of *pdxB*::Km^r mutant NU402 that grew on minimal salts medium plus 0.4% glucose (MMG) at 37°C were selected as follows. A 10-ml LBC culture of NU402 was grown at 37°C to mid-exponential phase, collected by centrifugation, suspended in 10 ml of MMG, centrifuged, and washed one more time. Washed cells were suspended in 10 ml of MMG, and 0.1 ml of cells was spread onto prewarmed MMG or MMG plus 0.5% (wt/vol) ACH (vitamin assay acid casein hydrolysate) plates, which were incubated at 37°C for 3.5 days. Pseudorevertants were restreaked onto the same selective medium at 37°C, and single colonies were saved by patching them onto LBC plates containing 50 μg of kanamycin per ml. Properties of two of three groups of *pdxB*::Km^r pseudorevertants were stable to repeated streaking on nonselective LBC medium plus 50 μg of kanamycin per ml at 37°C (see Table 3). Pseudorevertants of *serC*:: mini-Mu d mutants NU1107 and NU1113 could also be

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype[a]	Source or reference
E. coli K-12		
CAG12168	MG1655 *zgd-210*::Tn*10* (≃30% cotransducible with *serA*)	C. Gross (45)
CGSC2829 (formerly AB2829)	***aroA354 supE42*** λ$^+$	B. Bachmann collection
CGSC4539 (formerly AT3143)	*pdxC3 cycA1 cycB2 his-53 ilv-277 lacY29 met-65 proC24 purE41 pyrF30 rpsL97 tonA32 tsx63 xyl14* λ$^+$	B. Bachmann collection
CGSC4297 (formerly KL282)	***serC13*** *ompF627 pit10 phoA4*(Am) *relA1* (*serS14serS16*) *spoT1 supD32 tonA32* T2^r	B. Bachmann collection
GS415	***serA25*** *zgb224*::Tn*10 supE44 gal-6 lacY1 malA1 mtl-2 thi-1 tonA2 xyl-7* λ$^{r/-}$	G. Stauffer collection
JC7623	***recB21 recC22 sbc15*** *ara arg his leu pro thr*	A. J. Clark collection
NU402	W3110 *pdxB*::<Km^r(*Hin*dIII)	Laboratory stock (1)
NU426[b]	W3110 *sup*(Am) prototroph (probably W1485E)	Laboratory stock (1)
NU816[b]	W3110 Δ*lacU169 tna-2 sup*^{0}	C. Yanofsky collection
NU934	PO1683 *srl*::Tn*10 recA*	PO1693 × P1*kc*(TT9813)
NU979	NU816 Mu c$^+$	Mu *c*$^+$ lysogen of NU816
NU988	POII1734D(pNU178)	Transformant
NU989	NU934(pNU178)	Transformant
NU1054[c]	JC7623 *serC*::<mini-Mu dI194	JC7623 × linear pNU194
NU1055[c]	JC7623 *serC*::<mini-Mu dI195	JC7623 × linear pNU195
NU1107	VJS433 *serC*::<mini-Mu dI194	VJS433 × P1*kc*(NU1054)
NU1113	VJS433 *serC*::<mini-Mu dI195	VJS433 × P1*kc*(NU1055)
NU1331[c]	JC7623 *serC*::<mini-Mu dII193	JV7623 × linear pNU193
NU1333	VJS433 *serC*::<mini-Mu dII193	VJS433 × P1*kc*(NU1331)
POI1734 (also called PO1683)	F$^-$ *araD139 ara*::(Mu cts)3 Δ(*proAB argF lacIPOZYA*) *XIII rpsL* **Mu dΔ(*nerA,nerB*)dI1734 (Km^r *trp'A'-W209-lacZYA*)**	M. Casadaban (10)
POII1734D	F$^-$ *araD139 ara*::(Mu cts)3 Δ(*srl-recA*)::Tn*10* Δ(*lacIPOZYA*)*X74 galKE rpsL* **MuΔ(*nerA,nerB*)dII1734 (Km^r *lacZYA*)**	M. Casadaban (10)
TT9813	*metF*(Am) *eda-50* Δ*lacU169 rpsL136 thi-1 ara-14 mtl-1 xyl-5 tsx-78 tonA31* ***srl*::Tn*10 recA1***	J. Roth collection
VJS433	Δ(*argF-lac*)*U169* Δ(*trpEA*)*2*	V. Stewart collection
E. coli B or *E. coli* K-12/B		
WG5 (*E. coli* B)	*pdxF5*	W. Dempsey (14)
WG532 (*E. coli* K-12/B)	*pdxF5 lac rpsL*	W. Dempsey (14)
Plasmids		
pBR322	ColE1 replicon; Ap^r Tc^r	Laboratory stock (8)
pGT-17	*serA*$^+$ clone in pBR325; Ap^r Cm^r	G. Grant (51)
pNU169	pBR322 containing 12-kb *serC*$^+$ *aroA*$^+$ *Hin*dIII insert; Ap^r	This work
pNU174[c]	pBR322 containing 4.7-kb *serC*$^+$ *aroA*$^+$ *Hin*dIII-*Sph*I fragment from pNU169; Ap^r	This work
pNU178[c]	pBR322 *serC*$^+$ minimal clone containing a 1.6-kb *Nde*I-*Nde*I fragment from pNU169 in the pBR322 *Sal*I site; Ap^r	This work
pNU193[c]	pNU178[*serC*::mini-Mu dII193]; Ap^r Km^r	This work
pNU194[c]	pNU178[*serC*::mini-Mu dI194]; Ap^r Km^r	This work
pNU195[c]	pNU178[*serC*::mini-Mu dI195]; Ap^r Km^r	This work
pSAWT	*serA*$^+$ fusion to P_{trc} promoter in pKK233-2; Ap^r	G. Grant (43)

[a] Relevant markers are in boldface type for strains with complicated genotypes. <mini-Mu d indicates that *lacZ'* in the insertion element is in the opposite direction to that of *serC* transcription. Likewise, <Km^r(*Hin*dIII) means that transcription of *kan* in the kanamycin resistance cassette insertion is opposite to that of *pdxB* transcription. Ap^r, Km^r, and Tc^r, Resistance to ampicillin, kanamycin, and tetracycline, respectively.

[b] Strains were single colony isolated several times to provide uniform backgrounds for isogenic strain construction. The strong amber suppressor activity in NU426 was noted since publication of reference 1 but does not affect previous or present interpretations. NU816 lacks nonsense suppressing activity (data not shown).

[c] Plasmids and locations of inserts are depicted in Fig. 3.

isolated by a similar procedure, except that MMG plates contained serine and the six aromatic compounds listed above. However, *serC*::mini-Mu d pseudorevertants were not analyzed further in this study.

Enzyme assays. Crystalline D-erythrose-4-phosphate was purified to apparent homogeneity by paper chromatography as previously described (58) (see Results). Dehydrogenase assays were performed by measuring the change in absorbance at 340 nm (dA_{340}) per minute (dt) using a double-beam Shimadzu UV160 recording spectrophotometer fitted with an automatic sample changer. Crude cellular extracts for dehydrogenase assays were prepared as previously described (5) with the following exceptions. (i) Samples of only 5 ml were taken from exponentially growing cultures. (ii) Cells were washed, suspended, and subjected to sonic disruption in 5 ml of 0.2 M Tris hydrochloride–0.02 M $MgCl_2$ (pH 8.0). (iii) Resuspended cells were frozen and thawed once before sonic disruption. (iv) Sonic disruption was carried out by using the microprobe of a Branson Sonifier 250 for 2 min at power setting 3 and a 50% duty cycle. Reaction mixtures had the same composition as previously described (5), except that they contained 1.25 mM purified erythrose-4-phosphate or 1.0 mM glucose-6-phosphate as the substrate and both NAD^+ and $NADP^+$ as cofactors at

final concentrations of 1 mg/ml each. Protein concentrations in extracts were determined by the Bradford method, using bovine serum albumin as the standard (Bio-Rad Chemicals, Richmond, Calif.). Specific activities are expressed relative to control reaction mixtures that lacked substrate. Other control reactions established that the substrates alone did not contribute to dA_{340}/dt values. Enzyme assays for β-galactosidase were performed on chloroform-sodium dodecyl sulfate-permeabilized cells as described by Miller (35).

RESULTS

Effect of inhibitors of SerA activity or expression on pyridoxine synthesis in *pdxB* mutants. An unusual property of *pdxB* cassette insertion mutants is their ability to grow on MMG medium at 30°C (1). Because cassette insertions are stable and cannot transpose, growth of these *pdxB* mutants is most likely caused by a low-level alternate pathway that can synthesize sufficient pyridoxine at 30°C, but not at 37 or 42°C. In previous experiments, we showed that this alternate pathway for *pdxB* function was inhibited by addition of ACH to the MMG medium (1).

If the parallel scheme shown in Fig. 1 is correct, then we might expect SerA to replace PdxB in the alternate pathway of pyridoxine biosynthesis. To test this hypothesis, we determined the amino acid sensitivity of the alternate pyridoxine biosynthetic pathway. Table 2 shows that only valine, serine, glycine, isoleucine, and threonine inhibited the growth of *pdxB* mutants on MMG medium at 30°C. Inhibition of *E. coli* K-12 by valine is a general effect that is understood (53). Inhibition by serine, glycine, isoleucine, and threonine is exactly the pattern expected for decreased SerA enzyme activity or amount, because the former two amino acids are known to feedback inhibit the SerA enzyme (47), while the latter two cause reduced expression of *serA* (34). Inhibition by serine appeared to be qualitatively greater than inhibition by glycine, as evidenced by slight growth in heavily streaked areas on plates containing 0.13 mM glycine but equal or less growth on plates containing 0.04 mM L-serine (Table 2). Greater apparent inhibition by L-serine compared with glycine again is consistent with the known feedback inhibition characteristics of the SerA enzyme (38, 48, 54), although this interpretation does depend on relative uptake. Together, these highly specific inhibition patterns suggest a role for the SerA enzyme in the alternate pathway of pyridoxine biosynthesis and lend support to the scheme in Fig. 1.

Inhibition experiments like the one shown in Table 2 gave insight into other properties of the alternate, and indirectly, the major pathways of pyridoxine biosynthesis. When glycerol replaced glucose as the carbon source in the minimal salts medium (MM), growth of *pdxB* mutants was significantly reduced at 30°C, and only very tiny colonies appeared on plates after several days of incubation (Table 2). This observation is consistent with utilization of erythrose- and erythronate-4-phosphate as precursors to 4-hydroxythreonine, because glycerol must go through gluconeogenesis and the pentose phosphate pathway before it can be converted into four-carbon intermediates (see Discussion). Finally, it was not possible to test the proposed pathway by using a *pdxB serA* double mutant, because the double mutant requires serine, which inhibits the alternate pyridoxine pathway (Table 2). Therefore, lack of growth of a *pdxB serA* double mutant without added pyridoxine at 30°C cannot be interpreted to support a role for SerA in the alternate pyridoxine pathway.

TABLE 2. Effect of L-serine, glycine, and other amino acids on the growth of *pdxB*::Km^r mutants at 30°C

Supplement[a]	Colony growth[b]	
	Glucose	Glycerol
None	+	+/−
10^{-3} mM POX + PAL	++++	+++
0.04 mM L-Ser	+/−	NT
0.4 mM L-Ser	−	NT
4.0 mM L-Ser	−	−
4.0 mM L-Ser + 10^{-3} mM POX + PAL	++++	+++
0.13 mM Gly	+/−	−
0.47 mM D-Ala[c]	++	−
0.47 mM L-Ala[c]	++	−
0.47 mM D-Ala + 4.0 mM L-Ser	−	−
0.3 mM L-Val	−	NT
0.3 mM L-Ile	−	NT
0.3 mM L-Ile + 10^{-3} mM PAL	++++	NT
0.3 mM L-Thr	−	NT
Other amino acids or DAP[d]	+	NT

[a] Plates contained 1XE minimal medium, 0.4% (wt/vol) glucose or 0.4% (vol/vol) glycerol, and the indicated supplements, which were at concentrations suggested by Davis et al. (12), except for pyridoxine (POX) and pyridoxal (PAL) (13), and 0.04 and 0.4 mM L-Ser. Other amino acids were L-Pro, L-Asn, L-Trp, L-Asp, L-His, L-Leu, L-Phe, L-Met, L-Lys, L-Tyr, L-Arg, L-Gln, L-Cys, or L-Glu. DAP, DL-α, ε-Diaminopimelic acid.

[b] Overnight cultures of NU402 (*pdxB*::Km^r) grown in LBC medium plus 50 μg of kanamycin per ml at 37°C were centrifuged and washed twice in 1XE minimal salts medium plus 0.4% glucose. Washed cells were resuspended and then streaked onto prewarmed plates, and the plates were incubated at 30°C for 4 days before being scored. ++++ and +++, Large, opaque (normal) single colonies; ++, medium, opaque single colonies; +, small, transparent, mucoid single colonies (1); +/−, no single colonies, but some growth in the heaviest area of the streak (growth on 0.13 mM Gly or 0.04 mM L-Ser); −, no growth; NT, not tested.

[c] Besides pyridoxine and pyridoxal, only D-Ala or L-Ala supported growth at 37°C. Growth on D-Ala or L-Ala was still inhibited by L-Ser at 37°C.

[d] Colony size was considerably smaller on plates supplemented with L-Glu compared with other compounds.

Previously, we reported that pseudorevertants of *pdxB* cassette insertion mutants appear that can grow on MMG plates at 37 or 42°C (1). Similar to their *pdxB*::Km^r parent, *pdxB* pseudorevertants isolated on MMG medium were inhibited by ACH or serine and failed to utilize glycerol effectively as a carbon source (Table 3). Interestingly, these mutants fell into two classes; group 1 mutants grew well when pyridoxine and serine were added, whereas group 2 mutants were still inhibited by serine even in the presence of pyridoxine. A third pseudorevertant class, which was still inhibited by serine, was isolated on MMG plus ACH medium (Table 3). However, none of these *pdxB* pseudorevertants had normal growth properties, because all classes failed to grow in liquid MMG medium, even after 4 days of incubation at 37 or 42°C (data not shown). We were unable to demonstrate cotransduction between group 1 and 2 second-site mutations and a Tn*10*(Tet^r) marker in strain CAG12168 that cotransduces about 30% with *serA* (Table 1; also data not shown); therefore, the second-site mutation(s) acquired by this set of *pdxB* mutants do not appear to be in *serA*. This finding does not, however, contradict the scheme in Fig. 1, because a second possible pathway involving an aldolase-type condensation between glycolaldehyde and glycine could lead to 4-hydroxythreonine formation in *pdxB* pseudorevertants (Fig. 1, right top). This second alternate pathway will be described later in the section about growth properties of *serC* and *pdxB* insertion mutants.

We performed one additional experiment to test the relationship between *pdxB* and *serA* in pyridoxine biosynthesis. We reasoned that overexpression of SerA from recombinant

TABLE 3. Growth properties of NU402 (*pdxB*::Km^r) pseudorevertants at 37°C

Source of mutant[a]	Carbon source	Supplement[b]					
		None	L-Ser	POX + PAL	POX + PAL + L-Ser	ACH + Trp	ACH + Trp + L-Ser
MM + glucose (group 1)	Glucose	+	−	+++	+++	−	−
	Glycerol	−	−	NT	NT	−	−
MM + glucose (group 2)	Glucose	++	−	+++	+/−	−	−
	Glycerol	−	−	NT	NT	−	−
MM + glucose + ACH + Trp (group 3)	Glucose	+	−	+++	+++	+++	+++
	Glycerol	−	−	NT	NT	+/−	+/−

[a] Pseudorevertants that grew on the indicated media at 37°C were isolated as described in Materials and Methods. Group 1 and 2 mutations did not map near *serA* (see text). Group 3 mutants were unstable when propagated repeatedly on LBC medium plus 50 μg of kanamycin per ml.

[b] Cells were streaked from LBC plus kanamycin (50 μg/ml) patch plates onto 1XE minimal salts plates containing 0.4% (wt/vol) glucose, 0.4% (vol/vol) glycerol, 4.0 mM L-Ser, 10^{-3} mM pyridoxine (POX) plus pyridoxal (PAL), 0.5% (wt/vol) ACH, and 0.1 mM L-Trp as indicated. Plates were incubated about 3.5 days at 37°C before being scored. +++, Large, opaque (normal) single colonies; groups 1 and 2 + or ++, medium, transparent, slightly mucoid single colonies; group 3 +, heterogeneous single colonies ranging from tiny to large mucoid; group 2 +/−, no growth until 5.5 days of incubation, then full growth; group 3 +/−, steady, slow growth; −, no growth; NT, not tested.

plasmids might allow *pdxB* mutants to grow on MMG medium at temperatures greater than 30°C. However, we found that this experiment could not be performed properly, because recombinant *serA*$^+$ plasmids pGT-17 and pSWAT (Table 1) (43) inhibited growth of strains GS415 (*serA25*), NU402 (*pdxB*::Km^r), NU426 (prototroph), and NU816 (prototroph) to various extents on MMG at 30 to 42°C (data not shown).

Detection of erythrose-4-phosphate dehydrogenase activity in *E. coli* K-12 extracts. The scheme in Fig. 1 predicts the presence of two dehydrogenase activities that convert erythrose-4-phosphate into 3-hydroxy-4-phosphohydroxy-α-ketobutyrate. Erythrose-4-phosphate is an important precursor to the aromatic amino acids and its metabolism has been studied (37), yet to our knowledge, no erythrose-4-phosphate dehydrogenase activity has been reported.

Erythrose-4-phosphate is the only precursor that is commercially available in the proposed pathway leading to 4-hydroxythreonine. However, this compound is contaminated with glucose-6-phosphate. Consequently, we purified erythrose-4-phosphate away from glucose-6-phosphate by a paper chromatographic procedure (58). The resulting material did not serve as a substrate for purified yeast glucose-6-phosphate dehydrogenase, which indicates that we successfully removed the contaminants. Dehydrogenase activity was measured as described in the Materials and Methods by changes in absorbance at 340 nm (dA_{340}) with the time (dt) in a reaction mixture that contained both NAD$^+$ and NADP$^+$ at final concentrations of 1.0 mg/ml each. We also tested the purified erythrose-4-phosphate for the presence of inhibitors by mixing it with pure glucose-6-phosphate and then measuring yeast glucose-6-phosphate dehydrogenase activity. We found that the addition of purified erythrose-4-phosphate did not inhibit the yeast enzyme activity. Finally, we could not detect any other contaminants in the purified erythrose-4-phosphate by paper chromatographic analysis (data not shown). Although we cannot completely rule out the presence of low levels of other sugar phosphates in our purified sample, we think that this possibility is unlikely.

We next prepared crude cellular extracts of a W3110 prototroph, NU426, and a *pdxB*::Km^r derivative, NU402, by a freeze-thaw and sonication procedure (see Materials and Methods) (34). Resuspension and reaction mixtures were based on ones used to assay glucose-6-phosphate dehydrogenase (see Materials and Methods) (5). When reaction mixtures from either strain contained both NAD$^+$ and NADP$^+$ at 1.0 mg/ml each and either 1.25 mM erythrose-4-phosphate or 1.0 mM glucose-6-phosphate, we measured specific activities of 0.45 or 0.02 dA_{340}/dt/mg of protein, respectively. Thus, *E. coli* K-12 extracts seem to contain a low, but clearly detectable, level of erythrose-4-phosphate dehydrogenase activity. Because we had an extremely limited amount of erythrose-4-phosphate to use as substrate, we were unable to optimize reaction conditions, which may, in part, account for why the dehydrogenase activity appeared to be relatively low. Nevertheless, for the point of this paper, at least one of the hypothesized dehydrogenase activities in Fig. 1 seems to be present in *E. coli* K-12 cells. In this regard, it should be stressed that the products of this dehydrogenase have not yet been characterized chemically, and the structures shown in Fig. 1 and 4 are hypothetical.

Construction and properties of *E. coli* K-12 *serC*::mini-Mu d insertion mutants. The proposed role of *serC*-encoded phosphoserine aminotransferase in pyridoxine biosynthesis has been somewhat controversial (44). SerC enzyme contains pyridoxal phosphate, so *serC* mutants that seem to require serine or pyridoxine may contain enzyme with decreased coenzyme affinity. Such "K_m mutations" have been found for several pyridoxal phosphate-containing enzymes (18). Two loci that cause an apparent requirement for pyridoxine or serine, designated *pdxC* and *pdxF* in *E. coli* K-12 and B, respectively, map very close to *serC* (4, 20); it has been assumed that these mutations are in *serC* (20, 22), but this has not been rigorously established genetically. In addition, *pdxC* and *pdxF* mutants isolated previously contain point mutations, and the possibility of leaky phenotypes complicates certain interpretations (16).

To obtain additional evidence for the pyridoxine pathway in Fig. 1, we wanted to reexamine certain growth properties of *E. coli* K-12 mutants containing the *pdxC3* mutation or the *serC13* mutation, which was reported to require both serine and pyridoxine (44). However, we found that the *serC13* mutant grew on supplemented MMG medium containing serine or pyridoxine at 30°C, whereas it required both serine and pyridoxine at 37°C (data not shown). Therefore, the *serC13* mutation is undoubtedly a temperature-sensitive K_m allele of *serC*. In crystal spot tests, the archetypal *pdxC* mutant seemed to require pyridoxine or serine at all temperatures and likewise probably contains a *serC* K_m mutation.

Because of these difficulties with *serC* and *pdxC* point mutants, we constructed stable insertion mutations in the *E. coli* K-12 *serC* gene. Duncan and Coggins (22) sequenced

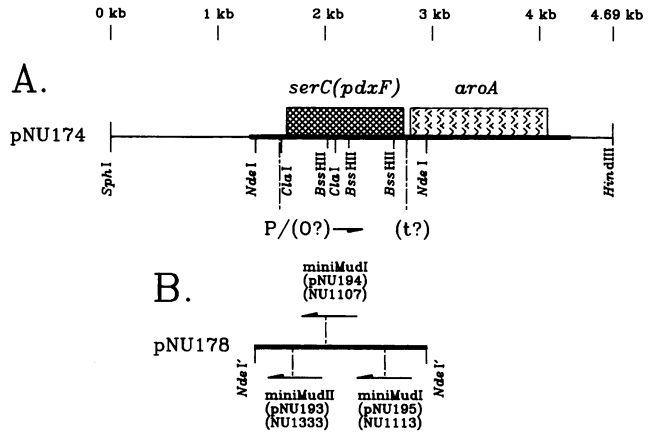

FIG. 2. Structure of the complex *serC-aroA* operon of *E. coli* K-12 showing positions of *serC*::mini-Mu d insertions. The figure is drawn to scale. Isolation of subclones pNU174 (A) and pNU178 (B) from plasmid pNU169 is described in Table 1 and Results. (A) Heavy black line, region sequenced in reference 22; hatched boxes, *serC* and *aroA* coding regions; P, mapped promoter; (O?) and (t?), putative operator and rho-independent terminator, respectively, deduced from the DNA sequence. (B) Minimal *serC* clone showing positions and designations of mini-Mu dI and mini-Mu dII insertions in pNU178 and the bacterial chromosome (see Table 1, Materials and Methods, and text). Arrows emphasize that the *serC* and *lacZ* reading frames are oriented in the opposite directions in all of the fusions. The *Nde*I' indicates that the site was altered during cloning.

serC and established that *serC* forms a complex operon with *aroA* (Fig. 2A). We used complementation of *pdxC* mutant CGSC4539 by a *Hin*dIII genomic library to isolate plasmid pNU169, which contains a 12-kb *serC*$^+$ *Hin*dIII-*Hin*dIII fragment identical to the one in the lambda-*aspC2* clone reported before (11). We constructed the two subclones shown in Fig. 2 from pNU169. On the basis of previous DNA sequence and transcription analyses (22), plasmid pNU178 is a minimal clone that contains only the *serC*$^+$ promoter and structural gene. We next caused mini-Mu dI and mini-Mu dII elements to jump into the three positions shown in Fig. 2B. In each case, the orientation of the *lacZ* coding sequence was opposite to that of *serC*. Finally, we linearized plasmids pNU193, pNU194, and pNU195, crossed the mini-Mu d elements into the chromosome of *recBC sbcB* mutant JC7623, and transduced the *serC*::mini-Mu d elements into Δ*lacU169* Δ*trpEA2* mutant VJS433 to give strains NU1333, NU1107, and NU1113, respectively (Table 1, Fig. 2).

Minimal *serC*$^+$ clone pNU178 complemented *serC*, *pdxC*, and *pdxF* mutations in strains CGSC4297, CGSC4539, WG5, and WG532 (Table 1), whereas mini-Mu d-containing plasmids pNU193, pNU194, and pNU195 did not complement these mutations (data not shown). Furthermore, *serC*::mini-Mu d insertion mutants NU1107 and NU1113 required serine, pyridoxine, tryptophan, phenylalanine, tyrosine, 4-aminobenzoate, 4-hydroxybenzoate, and 2,3-dihydroxybenzoate (Table 4). Together, these results establish conclusively that *serC* is required for both serine and pyridoxine biosynthesis and that *pdxC* and *pdxF* mutations are most likely alleles in *serC*. The conclusion that *serC*, *pdxC*, and *pdxF* are the same gene fits the fact that only one aminotransferase is probably in the pyridoxine biosynthetic pathway, because the pyridoxine ring contains a single nitrogen atom (Fig. 1).

The complete polarity of insertions at the beginning, middle, and end of *serC* on *aroA* expression (Table 4) indicates that there are no internal promoters upstream of *aroA* in *E. coli* K-12, an issue that was left unresolved by the DNA sequence analysis (22). Apparently the organization of the *serC-aroA* operon is similar in *Salmonella typhimurium*, because Tn*10* insertions in what is likely *serC* cause the same requirement for pyridoxine, serine, and the six aromatic compounds (30). Table 4 also shows that strain NU1333, which contains an insertion about one quarter into

TABLE 4. Growth properties of *serC*::mini-Mu d insertion mutants

Supplement[a]	Growth properties of bacterial strain:[b]					
	NU1333		NU1107		NU1113	
	30°C	37°C	30°C	37°C	30°C	37°C
None	−	−	−	−	−	−
L-Trp	−	−	−	−	−	−
POX + L-Trp	−	−	−	−	−	−
L-Ser + L-Trp	−	−	−	−	−	−
6ARO	−	−	−	−	−	−
POX + L-Ser + L-Trp	−	−	−	−	−	−
POX + 6ARO	−	−	−	−	−	−
L-Ser + 6ARO	+/−	+/−	−	−	−	−
ACH + L-Trp	+/−	+/−	−	−	−	−
L-Ser + POX + 6ARO	+++	+++	+++	+++	+++	+++
ACH + L-Trp + POX	+++	+++	+++	+++	+++	+++
Glycol + L-Ser + 6ARO	+++	+++	+++	+++	+++	+++
βHP + L-Ser + 6ARO	+++	+++	+++	+++	+++	+++
(0.1)βHP + L-Ser + 6ARO	++	++	+	+	+	+
D-Ala + L-Ser + 6ARO	−	−	−	−	−	−

[a] Cells were streaked from LBC plus kanamycin (50 μg/ml) patch plates onto prewarmed 1XE minimal salts plus 0.4% (wt/vol) glucose plates containing supplements at the following concentrations: 0.1 mM L-Trp; 5×10^{-4} mM pyridoxine (POX); 4.0 mM L-Ser; 6 aromatic compounds (6ARO) (0.1 mM L-Trp, 0.1 mM L-Tyr, 0.3 mM L-Phe, and 10^{-3} [each] 4-aminobenzoate, 4-hydroxybenzoate, and 2,3-dihydroxybenzoate); 0.5% (wt/vol) ACH; 0.1 mM glycolaldehyde (Glyol); 1.0 mM β-hydroxypyruvate (βHP). Plates were incubated 4 or 3 days at 30 or 37°C, respectively, before colonies were scored. The same results were obtained when patch plates were replica plated onto the supplemented minimal medium. +++, Large, opaque (normal) single colonies; ++, medium, opaque single colonies; +, small, opaque single colonies; +/−, tiny, but distinct opaque single colonies; −, no growth.

[b] Positions of mini-Mu d insertions are depicted in Fig. 2. Similar results were obtained in the NU816 (Δ*lac*) genetic background. NU1107 and NU1113 formed light blue patches on LBC plus kanamycin plus 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates, whereas NU1333 formed white patches (see text).

serC (Fig. 2B), retains enough partial enzymatic activity to grow slowly in the absence of pyridoxine but not serine. Presumably, the amino terminus of phosphoserine aminotransferase is not strictly essential for enzymatic activity. Finally, we noticed that the *lacZ* transcriptional fusions in strains NU1107 and NU1113, which are in the opposite orientation to that of *serC* and *aroA*, expressed a low, but detectable, level of β-galactosidase activity (≈1 Miller unit) in cells grown in LBC medium. Therefore, antisense transcription seems to occur in the *serC-aroA* operon. Presently, we do not know whether this antisense transcription plays a regulatory function or whether its level changes; however, the opposite strand near the *serC-aroA* intercistronic region does contain sequences reminiscent of a Tyr box (37) and several σ^{28} consensus recognition sequences (data not shown) (6).

Growth properties of *serC*, *pdxB*, and *pdxA* insertion mutants. Previously, several groups showed that glycolaldehyde can replace pyridoxine as a supplement to allow *pdxB* mutants to grow on MMG medium (36, 49, 50). However, it was also asserted that in order for *pdxB* mutants of *E. coli* B to be supplemented by glycolaldehyde, they need to acquire additional second-site mutations (19). Our recent results do not support these assertions for *E. coli* K-12 strains, since we find that *pdxB*::Km^r insertion mutants, which have never been exposed to selection for growth without pyridoxine, grow normally on MMG medium plus glycolaldehyde at 37 and 42°C (1).

Figure 1 illustrates how glycolaldehyde can supplement *pdxB* mutants in the absence of pyridoxine. Isotope labeling experiments establish firmly that glycolaldehyde is not a precursor in the major pyridoxine biosynthetic pathway (56); however, added glycolaldehyde can provide an alternate pathway, probably by condensing with glycine to form 4-hydroxythreonine (19). If the scheme in Fig. 1 is correct and *pdxB* and *serC* function in the same branch of the pyridoxine pathway, then glycolaldehyde should supplement *serC* mutants as well as *pdxB* mutants. The full growth shown in Fig. 3B and Table 4 of *serC*::mini-Mu d insertion mutants in medium supplemented with 0.1 mM glycolaldehyde confirms this prediction. This finding is different from previous conclusions about the effect of glycolaldehyde on *serC* mutants, because these earlier studies again asserted that second-site mutations had to occur in *serC* mutants before they could use glycolaldehyde as a replacement for pyridoxine (19). When we streaked our *serC* insertion mutants, which had never been grown without pyridoxine, onto MMG or MM plus glycerol plates containing serine, the six aromatic compounds, and glycolaldehyde, they grew readily (Table 4) (data not shown). Therefore, we conclude that *pdxB* and *serC* are on the same branch of the pyridoxine pathway. By contrast, *pdxA* insertion mutants are not supplemented by glycolaldehyde (15), and this gene likely mediates a different branch of the pyridoxine pathway, which is again consistent with Fig. 1 (see Fig. 4 and Discussion).

Besides glycolaldehyde, several other compounds have been implicated as possible precursors of 4-hydroxythreonine and pyridoxine (for a review, see reference 29). The scheme in Fig. 1 clearly accounts for an involvement of glutamate in pyridoxine biosynthesis (15), since glutamate is the amino donor for the SerC enzyme. By using crystal spot tests, streaking the bacteria onto supplemented MMG and MM plus glycerol plates, and growing them in supplemented liquid minimal medium (see Materials and Methods), we also tested whether β-hydroxypyruvate, D-alanine, L-alanine,

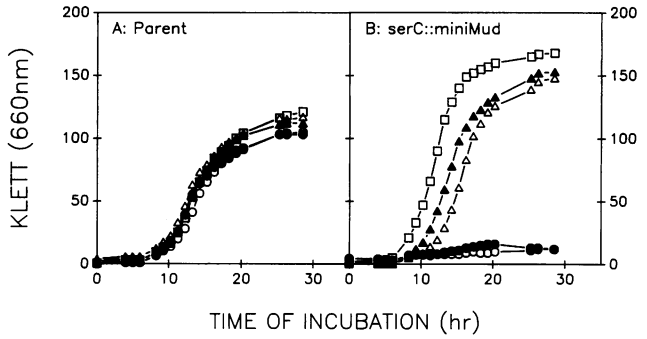

FIG. 3. Growth curves of *serC*$^+$ parent NU1056 (A) and *serC*::mini-Mu dI mutant NU1107 (B) in supplemented minimal salts medium containing pyridoxine, glycolaldehyde, or β-hydroxypyruvate. Overnight cultures (5 ml) grown at 37°C in LBC medium plus 50 μg of kanamycin per ml were collected by centrifugation, washed twice in 1XE minimal salts medium plus 0.4% glucose (MMG), and resuspended in 5 ml of MMG. A 0.1-ml sample of washed cells was used to inoculate a Nephelo flask containing 10 ml of MMG plus 4.0 mM L-Ser plus 6 aromatic compounds (0.1 mM L-Trp, 0.1 mM L-Tyr, 0.3 mM L-Phe, and 10^{-3} mM each of 4-aminobenzoate, 4-hydroxybenzoate, and 2,3-dihydroxybenzoate), and final concentrations of the compounds noted below. Culture flasks were vigorously shaken at 37°C, and turbidities were measured at the indicated times after inoculation. Symbols: ○, no addition; □, 100 ng of pyridoxine per ml; ▲, 0.1 mM glycolaldehyde; ●, 0.1 mM β-hydroxypyruvate; △, 1.0 mM β-hydroxypyruvate.

glycolate, or glycine would support growth of *pdxB*::Km^r or *serC*::mini-Mu d insertion mutants at temperatures between 30 and 42°C (Fig. 3, Tables 2 and 4). Figure 3B shows that high (1.0 mM), but not intermediate (0.1 mM), concentrations of β-hydroxypyruvate allowed nearly full growth of *serC*::mini-Mu d mutants in supplemented minimal medium. This effect was detected more clearly in liquid cultures (Fig. 3) than on plates (Table 4), because after 4 days of incubation, there was residual growth on plates containing 0.1 mM β-hydroxypyruvate. For comparison, the concentration of pyridoxine or glycolaldehyde required to attain normal growth of *serC* mutants in liquid medium was 5×10^{-4} mM or 0.1 mM, respectively (Fig. 3B). By contrast, high concentrations of β-hydroxypyruvate failed to support the growth of *pdxA*::Km^r or *pdxB*::Km^r mutants (data not shown). This differential effect of high concentrations of β-hydroxypyruvate on the growth of *serC* and *pdxB* mutants will be discussed further below (see Discussion).

Another apparent differential growth effect between *pdxB* and *serC* mutants was observed in supplemented minimal medium containing D-alanine or L-alanine. Previously, we reported that *pdxB*::Km^r mutants would grow at 37°C on MMG plates supplemented with D-alanine instead of pyridoxine (1). In additional experiments, we found the following results. (i) L-Alanine could replace D-alanine to allow growth of *pdxB*::Km^r mutants on MMG solid medium. (ii) Alanine-supplemented growth occurred only on solid medium but not in liquid medium. (iii) D-Alanine could not replace pyridoxine in MM plus glycerol medium. (iv) The addition of serine prevented growth of *pdxB*::Km^r mutants on MMG plus alanine medium (Table 2). These results are consistent with our previous explanation that the addition of D-alanine, which is likely the product of a pyridoxal phosphate containing racemase, relieves pressure on the alternate pathway of pyridoxine biosynthesis, and sufficient pyridoxine is synthesized to allow residual growth at higher temperatures (1). We also found that D-alanine failed to

support growth of *serC*::mini-Mu d mutants on MMG plus serine plus aromatic compounds medium at 37°C (Table 4). However, this apparent difference between the *pdxB* and *serC* mutants is expected; *serC* mutants require serine for growth, but serine inhibits the alternate pathway of pyridoxine biosynthesis.

In a final series of supplementation tests, we found that neither glycolate nor glycine could replace pyridoxine for growth of *pdxB*::Km^r or *serC*:mini-Mu d mutants on supplemented MMG medium at 37°C (data not shown). This result contradicts the previous contention that *pdxB* encodes glycolate dehydrogenase, which converts glycolate into glycolaldehyde (50); for if this were correct, then the *serC* mutant should have grown on medium containing glycolate. Lack of growth on glycine medium may imply that sufficient glycolaldehyde is not normally present in *E. coli* K-12 cells to allow stimulation of the alternate pathway depicted in Fig. 1 by glycine addition.

DISCUSSION

We propose a pathway for one branch leading to the biosynthesis of pyridoxine (vitamin B_6) in *E. coli* K-12 (Fig. 1). This pathway produces the hypothetical intermediate, 4-hydroxythreonine, and is based upon a parallelism to the phosphorylated pathway of serine biosynthesis (47). Evidence for such a parallel pathway comes from our previous finding that the PdxB and SerA enzymes are homologs (42). In fact, these two enzymes seem to represent an interesting new family of 2-hydroxyacid dehydrogenases (24). This functional homology, combined with the structural similarity between 4-hydroxythreonine and serine and the likely involvement of the SerC enzyme in pyridoxine biosynthesis, suggested the pathway shown in Fig. 1.

This hypothetical pathway leads to several inferences that are tested by experiments in this paper. If PdxB and SerA are evolutionarily and functionally related, then residual activity of SerA in the pyridoxine pathway might account for a low-level, alternate pathway of pyridoxine biosynthesis detected in *pdxB*::Km^r mutants at 30°C (1). The highly specific inhibition patterns of the alternate pyridoxine pathway presented in Tables 2 and 3 is exactly the one expected for inhibition of SerA enzymatic activity or *serA* expression. Moreover, the results in Table 3 imply that a serine-sensitive pathway is required, at least in part, even for growth of *pdxB* pseudorevertants. Another prediction of the scheme in Fig. 1 is the presence of erythrose-4-phosphate dehydrogenase activity in *E. coli* K-12. We show here that such an activity can indeed be detected at a low level in crude cellular extracts. One more inference of Fig. 1 is that *pdxB* and *serC* mediate steps in the same branch of the pathway. By constructing chromosomal insertion mutations at three positions in *serC*, we confirmed that this gene is required for both serine and pyridoxine synthesis, without the need to acquire second-site mutations in some unspecified gene (Table 4). We also show that *pdxC* and *pdxF* mutations are located in *serC*, which means that the separate listing of *pdxC* in the *E. coli* K-12 linkage map is incorrect (4). Finally, by considering the growth responses of *pdxA*, *pdxB*, and *serC* insertion mutants on glycolaldehyde (Fig. 3) (40), we conclude that *pdxB* and *serC*, but not *pdxA*, likely play roles in the same branch of the pyridoxine pathway. Taken together, these observations all support the hypothetical pathway shown in Fig. 1.

Of course, isotope labeling experiments are required for rigorous proof of a biosynthetic pathway. Fortunately, careful tracer experiments have already been performed for pyridoxine biosynthesis by Hill and co-workers (27–29). For a variety of reasons, these experiments did not yield a definitive pathway; however, any proposed pathway must account for the labeling patterns they observed. To understand the labeling patterns, it is necessary to fit Fig. 1 into general intermediary metabolism, as shown in Fig. 4. In this context, it becomes clear that labeled glycerol units are carried through gluconeogenesis and then the pentose phosphate pathway before they are converted into erythrose-4-phosphate. In previous studies, the carbon and hydrogen atoms of glycerol labeled at several different positions have been traced into specific positions of pyridoxine (27, 29, 56, 57). In particular, carbons 5, 5′, and 6 seem to be derived from an intact triose unit (27). As noted by Dempsey (19), these data are still consistent with incorporation of the glycerol backbone into a four-carbon unit, followed by a decarboxylation. When we followed specifically labeled glycerol molecules through the pathways abbreviated in Fig. 4, we found that the labeling patterns are completely consistent with the hypothetical pathway shown in Fig. 1 (analysis not shown). In addition, the pathway in Fig. 1 accounts for the finding that glutamate is required for pyridoxine biosynthesis, but its carbon backbone is not incorporated into the pyridoxine ring (15, 17). Finally, labeling patterns of pyridoxine isolated from *pdxB* mutants grown in labeled glycine or glycolaldehyde agree with predictions based on Fig. 1 and 4 (26, 31). Thus, the hypothetical pathway in Fig. 1 leading to nitrogen 1 and carbons 5, 5′, and 6 of the pyridoxine ring is supported completely by an extensive body of isotope tracer data (17, 26, 27, 29, 31, 56, 57).

The extended pathway in Fig. 4 can also account for several other observations related to pyridoxine biosynthesis. This scheme explains why thiamine (vitamin B_1) is required for pyridoxine synthesis (15), since thiamine is essential for the transketolase activities in the pentose phosphate pathway used in erythrose-4-phosphate biosynthesis (23). In Fig. 1 and 4, we assume that the intermediates are phosphorylated, as they are in the serine pathway. This reasonable assumption and the well-known inability of phosphorylated compounds to leave bacterial cells would account for the inconclusiveness of mutant cross feeding experiments noted previously and attempted by us (data not shown) (21). Presently, there is no direct evidence for involvement of a phosphatase analogous to SerB (Fig. 1 and 4) in pyridoxine biosynthesis. However, the function of *pdxJ* is unknown; it is possible that a nonspecific phosphatase may convert 4-phosphohydroxythreonine into 4-hydroxythreonine. At any rate, there is precedent for the phosphorylated pathway proposed in Fig. 1 and 4 in erythritol catabolism in *Brucella abortus* (46).

The differential growth of *serC*::mini-Mu d, but not *pdxB*::Km^r, mutants on medium containing high concentrations of β-hydroxypyruvate (Fig. 3; Table 4) can also be explained by reference to Fig. 4. Vastly more ($>10^4$) β-hydroxypyruvate than pyridoxine is required to support growth of *serC* insertion mutants (Fig. 3B), which is consistent with the notion that β-hydroxypyruvate is not a normal precursor of pyridoxine (36). Unfortunately, tracer data do not yet exist for the incorporation of β-hydroxypyruvate into pyridoxine (29), so several assumptions need to be made. We assume that when excess exogenous β-hydroxypyruvate is added to *pdxB* mutants, a low-level activity of the SerC enzyme converts it into serine (44); therefore, β-hydroxypyruvate does not accumulate in *pdxB* mutants. By contrast, when added in excess to *serC* mutants, β-hydroxypyruvate

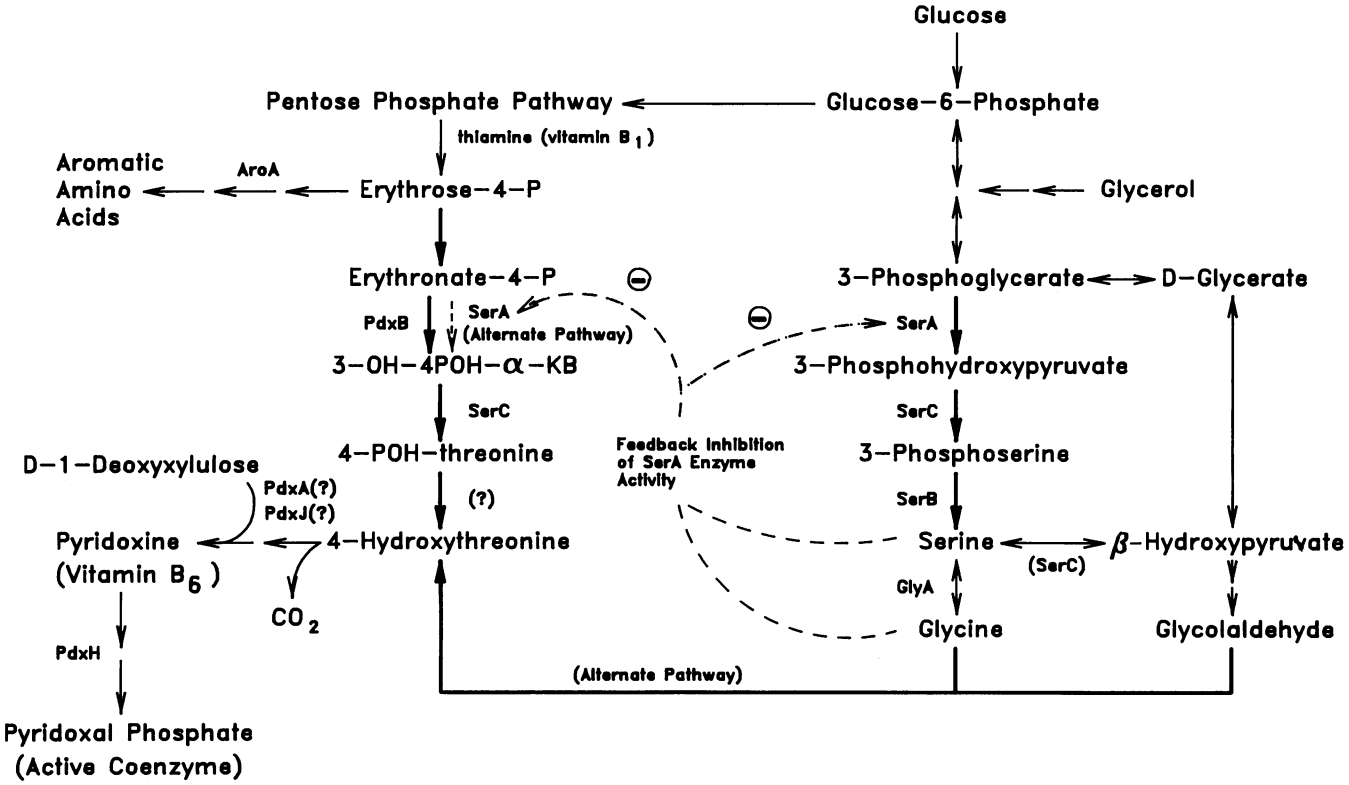

FIG. 4. Proposed *pdxB-serC* mediated branch of the pyridoxine biosynthetic pathway in the context of general intermediary metabolism. Steps not marked by an enzymatic activity may include more than one biosynthetic step. The pathways in Fig. 1 are condensed, abbreviated, and represented with heavy arrows near the center of the figure. Feedback inhibition of SerA enzyme activity is indicated by broken lines. Other features of the extended pathway are described in the text. The exact roles of *pdxA* and *pdxJ* are unknown, although *pdxA* is not on the *pdxB-serC* branch of the pathway (see text). The *pdxH* gene most likely encodes pyridoxine phosphate oxidase (19).

accumulates intracellularly and is eventually converted into "activated glycolaldehyde" by a decarboxylation analogous to the one detected in yeast cells (25, 39). The activated glycolaldehyde can then be converted into glycolaldehyde, which acts in the alternate pathway of pyridoxine biosynthesis (Fig. 1 and 4). Besides this reaction scheme, glycolaldehyde can be synthesized as a byproduct of folate biosynthesis (9) and D-arabinose catabolism (33).

The existence of normally dormant pathways leading to glycolaldehyde synthesis can account for the appearance of pseudorevertants of *pdxB* and *serC* insertion mutants (Table 3; also data not shown). As mentioned in Results, second-site mutations in *pdxB* pseudorevertants did not map to *serA*; however, these suppressor mutations might activate the pathway leading to glycolaldehyde indicated in Fig. 4. Even with this activation, the *serA*-mediated alternate pathway probably still contributes to pyridoxine synthesis, since growth of *pdxB* pseudorevertants was inhibited by serine (Table 3). Partial contribution of the *serA* alternate pathway could also account for the inability of *pdxB* pseudorevertants to grow on MM plus glycerol plates, because glycerol should not be an efficient carbon source for four-carbon compounds like erythrose-4-phosphate (Fig. 4). Alternatively, an activated pathway leading directly to glycolaldehyde synthesis might itself be inhibited by serine.

The finding that *pdxB* and *serA* are homologs (42) and the two pathways share SerC enzyme function (Fig. 1, 3, and 4; Table 4) suggests an evolutionary scenario in which the *pdxB-serC* branch of pyridoxine biosynthesis arose by gene recruitment from the serine pathway (42). In this regard, it is interesting that SerA enzyme seems still to retain residual activity in the pyridoxine pathway (Tables 2 and 3). Such low-level activity is a prerequisite in current models of how gene recruitment occurs (32). By contrast, SerC enzyme seems to function in both pathways by using different substrates (Fig. 1 and 4). Yet, it appears unlikely that these two activities are contained in different domains of the SerC protein, since there is an extraordinary degree of conservation between bacterial and mammalian phosphoserine aminotransferase (55). If the bacterial SerC enzyme had two domains, then it is improbable that they would be conserved in the mammalian enzyme, because mammals do not synthesize pyridoxine. Enzymological characterization of the PdxB, SerC, and SerA enzymes, physiological analysis of the *pdxJ* gene, and additional isotope tracer experiments are needed to test rigorously the pyridoxine biosynthetic pathway postulated here.

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