2-Deoxyribose Gene-Enzyme Complex in *Salmonella typhimurium*: Regulation of Phosphodeoxyribomutase

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Received for publication 5 December 1968

Phosphodeoxyribomutase, the enzyme which catalyzes the interconversion of 2-deoxyribose-1-phosphate to 2-deoxyribose-5-phosphate, has been partially purified from *Salmonella typhimurium*. The enzyme had an absolute requirement for manganese ion and was stimulated by glucose-1, 6-diphosphate. Phosphodeoxyribomutase was induced by deoxyribose-5-phosphate and was coordinately regulated with the enzymes thymidine phosphorylase and deoxyribose-5-phosphate aldolase, type II. Mutants deficient in these three enzymes were isolated and mapped close to the threonine locus in *S. typhimurium*. The three enzymes thymidine phosphorylase, deoxyribose-5-phosphate aldolase, type II, and phosphodeoxyribomutase are controlled by a series of linked genes and appear to constitute an operon.

*Salmonella typhimurium* LT2 can utilize 2-deoxyribose as a sole carbon and energy source (2, 3, 12). When this organism is grown in the presence of 2-deoxyribose, there is an increase in the levels of the following enzymes: deoxyribose kinase; deoxyribose-5-phosphate aldolase (2-deoxy-D-ribose-5-phosphate acetaldehyde lyase, EC 4.1.2.4); thymidine phosphorylase (thymidyl deoxygenase; 2-deoxyribosyltransferase, EC 2.4.2.4); and 1,5-phosphodeoxyribomutase (PDR mutase) (3, 12). In a previous report (12), Hoffee suggested on the basis of both biochemical and genetic evidence that in *Salmonella* there existed two genes under separate regulation which contained information for the synthesis of deoxyribose-5-phosphate aldolase (deoxyribose-5-P aldolase). These two enzymes were tentatively defined as type I and type II on the basis of their pattern of induction as compared with the induction of deoxyribose kinase.

Work with *Escherichia coli* (4, 5, 16, 21) has suggested that in this organism there is no genetic information for deoxyribose kinase. Owing to the absence of deoxyribose kinase, *E. coli* is incapable of growing on 2-deoxyribose as a carbon and energy source. However, *S. typhimurium* LT2 and *E. coli* appear to have the same metabolic pathway for deoxyribonucleoside (deo) catabolism, and the role of this pathway in thymine metabolism is similar in the two organisms. The deo pathway consists of the following enzymes: thymidine phosphorylase, which catalyzes the interconversion of thymidine to thymine and deoxyribose-1-phosphate; PDR mutase, which catalyzes the interconversion of deoxyribose-1-phosphate to deoxyribose-5-phosphate; and deoxyribose-5-P aldolase, which catalyzes the splitting of deoxyribose-5-phosphate to acetaldehyde and glyceraldehyde-3-phosphate (12, 16). It has been known for some time that thymine-requiring mutants isolated by the aminopterin selection procedure of Okada et al. (20) show a high thymine requirement for growth, i.e., 20 µg/ml. Secondary mutants have been reported which are capable of growing on lower levels of thymine, i.e., 1 to 2 µg/ml (1, 10). Breitman and Bradford and others (2, 4, 5, 16, 21) have shown with *E. coli* that these low thymine-requiring mutants lack either deoxyribose-5-P aldolase or PDR mutase. This deficiency could result in the accumulation of deoxyribose-1-phosphate, a necessary co-substrate for the conversion of thymine to thymidylate. Thus, deoxyribose-1-phosphate has been suggested as the limiting factor in thymine utilization in a mutant deficient in thymidylate synthetase (4, 16).

The low thymine requirement as well as the deo region have been mapped in *E. coli* (1, 7, 16) and shown to be co-transducible with the threonine marker (16). A recent report suggested that the deo region in *S. typhimurium* also maps in the threonine region but does not cotransduce with threonine (8). These results with *S. typhimurium* have been confirmed in this laboratory (see below).
In *S. typhimurium*, the enzymes of the deo pathway (16), i.e., thymidine phosphorylase, PDR mutase, and deoxyribose-5-P aldolase, can be induced by growth in the presence of thymidine or deoxyribose-5-phosphate, as well as in the presence of deoxyribose, suggesting that the inducer of this pathway is deoxyribose-5-phosphate (12). (In this paper, the deoxyribose-5-P aldolase which is induced by deoxyribose-5-phosphate and regulated with thymidine phosphorylase and PDR mutase will be defined as type II.) Similarly, in *E. coli* it has been suggested that the inducer of the deo pathway is a product of thymidine catabolism, namely deoxyribose-5-phosphate (2, 4, 5, 16).

This paper deals mainly with one of the enzymes in the deo pathway, PDR mutase, which reversibly catalyzes the conversion of deoxyribose-1-phosphate to deoxyribose-5-phosphate (14, 24). In this report, we present data on the partial purification of PDR mutase, some of its properties, and its regulation both in wild-type *S. typhimurium* and in some of the various types of thymine-requiring mutants of this organism. Preliminary studies on the genetic mapping of the deo region in *S. typhimurium* are also presented.

**MATERIALS AND METHODS**

**Bacterial strains.** The bacterial strains employed in this study and their phenotypes are listed in Table 1. Cultures were maintained on nutrient slants containing 20 µg of thymine per ml, except for thymidine phosphorylase-negative mutants which were maintained on nutrient slants containing 20 µg of thymidine per ml. Transduction studies were performed with the phage PTL22.

**Materials.** Commercial preparations were used unless otherwise specified. 2-Deoxyribose was obtained from International Chemical and Nuclear Corp., City of Industry, Calif. 2-Deoxyribose-5-phosphate, 2-deoxyribose-1-phosphate, glucose-1,6-diphosphate, and thymidine were purchased from Calbiochem, Los Angeles, Calif. Yeast alcohol dehydrogenase and glycerol phosphate dehydrogenase-triose phosphate isomerase mixtures were obtained from Boehringer and Sons, New York, N.Y. Fructose-diphosphate aldolase was prepared from rabbit muscle by the method of Taylor et al. (25).

**Culture and growth of organisms.** Cells were grown in Casamino Acids medium buffered with phosphate at pH 7, in Nutrient Broth (Difco), or in a synthetic medium of the following composition (per cent, w/v): KH₂PO₄ 0.3; K₂HPO₄ 0.7; (NH₄)₂SO₄ 0.1; MgSO₄ 0.001; and carbon sources as indicated. The carbon sources and salts were sterilized separately and added as concentrated solutions. Solid medium had 13 g of agar added per liter. Thymine or thymidine was added where indicated; high thymine medium contained 20 µg of thymine per ml and low thymine medium contained 2 µg of thymine per ml. Endo-deoxyribose plates are Endo plates (Difco) with 0.8% deoxyribose in place of lactose. Cell growth was followed in liquid media by measuring the increase in optical density on a Klett-Summerson colorimeter.

**Induction of enzymes.** Induction of the wild-type organism was accomplished by adding the inducer (2-deoxyribose or thymidine) to the cells during logarithmic growth in Casamino Acids medium and harvesting the cells when the inducer was 90% utilized. The thymine-requiring mutants unable to utilize thymidine or deoxyribose as carbon and energy sources were grown to log phase in Casamino Acids medium, and the inducer was added 4 hr prior to harvesting. The cells were washed in 10⁻⁴ M ethylenediaminetetraacetate (EDTA), pH 7.4, and stored at −10 C.

**Preparation of extracts.** Extracts of cells were made by grinding the frozen pellets with alumina (twice the wet weight of the cells). The mixture was suspended in 10⁻⁴ M EDTA plus 10⁻² M mercaptoethanol, pH 7.4, and centrifuged for 45 min at 27,000 × g. The supernatant fluids were assayed for enzyme activities and protein concentration.

**Enzyme assays.** Deoxyribose-5-P aldolase, deoxyribose kinase, and thymidine phosphorylase were assayed as previously described (12). PDR mutase was assayed spectrophotometrically by measuring the conversion of 2-deoxyribose-1-phosphate to 2-deoxyribose-5-phosphate and eventually to ethyl alcohol in the presence of 2-deoxyribose-1-phosphate, reduced nicotinamide adenine dinucleotide (NADH), deoxyribose-5-P aldolase, yeast alcohol dehydrogenase, glucose-1,6-diphosphate, and manganese ion. This assay couples the PDR mutase assay to the deoxyribose-5-P aldolase assay. Hoffee has shown that the aldolase assay is unaffected throughout a broad pH range from 7.3 to 8.4 and is not inhibited or enhanced by metals (13).

Fructose diphosphate aldolase was assayed spectrophotometrically by following the conversion of fructose diphosphate to glycerol phosphate in the presence of NADH, fructose diphosphate, potassium ion, glycerol phosphate dehydrogenase, and triose phosphate isomerase (22).

**Protein determination.** Protein was determined by the method of Lowry et al. (18) or that of Bucher (6), with fructose diphosphate aldolase purified from rabbit muscle as a standard.

**Isolation of thymine-requiring mutants.** Thymine-requiring mutants were isolated by a modification of the aminopterin method of Okada et al. (20), as previously described (12).

**Interrupted matings.** Interrupted-mating experiments were performed with the Hfr A strain of Zinder as a donor and a threonine-thymine-requiring double mutant of *S. typhimurium* (deoB4214) as the recipient. Selection for the threonine marker was made on minimal-glucose plates containing 20 µg of thymine per ml and for the PDR mutase marker on minimal-thymidine plates containing 50 µg of threonine per ml and 2 g of thymidine per liter. Cultures of Hfr A were grown in Nutrient Broth. Strain *deoB4214* was grown in Nutrient Broth containing thymine (10 µg/ml) plus threonine (50 µg/ml). After overnight incubation, the cultures were diluted 1:40 in the same medium and
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Table 1. Bacterial strains*

<table>
<thead>
<tr>
<th>Strain</th>
<th>his</th>
<th>gal</th>
<th>dhaA</th>
<th>dhaB</th>
<th>dhaC</th>
<th>dhaD</th>
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<th>deoB</th>
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* The following symbols are used: +, wild type; - , inability to synthesize or utilize as a carbon source; deo, deoxynucleoside; deoA, thymidine phosphorylase; deoB, PDR mutase; deoC, deoxyribose-5-P aldolase, type II; thy, thymine; gal, galactose; his, histidine; thr, threonine; drb, deoxyribose; drbA, deoxyribose permease; drbB, deoxyribose kinase; drbC, deoxyribose regulatory gene; drbD, deoxyribose-5-P aldolase, type I.

A prototrophic strain of S. typhimurium LT2 from which all mutants were derived was obtained from E. Englesberg.

An Hfr donor strain obtained from D. Kessler with an origin at 120 min on the Salmonella chromosome (23).

A deoB\( thr\) thy\(\) was isolated by plating a deoB\( +\) thr\( +\) thy\( +\) strain on minimal-glucose plates containing suboptimal levels of thymine. The threonine auxotroph was originally selected by the penicillin technique (9) in a thr\( +\) thy\( +\) strain isolated by the procedure of Okada et al. (20).

These mutants were isolated as low thymine-requiring mutants as previously described (12).

These mutants were isolated as low thymine-requiring mutants as previously described (12); deoC4006, deoC4007, and deoC4008 appear to be double mutants deficient in deoxyribose-5-P aldolase.

A mutant drbC4006-T is a strain (deoC4006) which has been transduced to deoC\( +\) deoB\( +\) by PT22 phage grown on deoB4012.

These strains are thymidine phosphorylase-negative strains isolated in the double mutant deoC4008. These mutants were selected by plating deoC4008 on Nutrient Agar containing 0.1% thymidine. Colonies arising on these plates were tested on minimal-glucose plates containing either 20 \(\mu\)g of thymine or thymidine per ml. Those growing on the latter but not the former were isolated and purified. These isolates were then tested for the absence of deoxyribose-5-P aldolase by testing for a negative reaction on Endo-deoxyribose plates.

grown to mid-log phase with gentle shaking at 37 C. About 2 \(\times\) 10\(^{10}\) donor cells and 10\(^6\) recipient cells were mixed on a membrane filter (25-mm diameter, 0.45-\(µ\)m pore size; Millipore Corp., Bedford, Mass.). The liquid was drawn through the filter, and the filter was placed on a nutrient soft-agar plate at 37 C for 5 min. The filter was transferred to 16 ml of Nutrient Broth (zero time) and incubated at 37 C with gentle agitation. As matings continued, 0.2-ml samples were removed at 3-min intervals and diluted with 0.8 ml of Nutrient Broth during the first 12 min. After 12 min, samples were removed at 2-min intervals. The samples were vigorously agitated for 10 sec on a sabre saw blender [a modification of the one proposed by Low and Wood (17)]. Portions of 0.1 ml were pipetted into duplicate tubes containing 2 ml of soft agar plus the appropriate additions, mixed, and poured onto hard-agar plates containing the same additions. After 2 days of incubation at 37 C, the recombinant colonies were counted. Growth of the donor cells was prevented by omitting from the minimal medium the supplements required by the auxotrophic strains.

Transduction studies. Transductional analyses were performed with overnight cultures of the various recipients and phage PT22 (11). The phage were added to 1 ml of culture at a multiplicity of infection of 5. The mixture was incubated for 6 min at 37 C, and then 0.1 ml samples were plated on the appropriate media. Transductants were tested for nonselected markers by picking and streaking to the various selective media.

RESULTS

Partial purification and properties of PDR mutase: growth of cells and extraction. Flasks (2-liter) containing 1 liter of Casamino Acids medium buffered at pH 7 with phosphate were inoculated with 25 ml of an overnight culture of wild-type S. typhimurium. The flasks were incu-
bated with shaking at 37 C for 4 hr, at which time the cells were in log phase (optical density in Klett units, 110); sterile 2-deoxyribose was then added to the cells to a final concentration of 0.2%. The cells were incubated at 37 C with aeration for an additional 2 hr, and then were rapidly chilled and harvested in a refrigerated Sorvall centrifuge (at the time of harvest, 90% of the 2-deoxyribose in the medium had been used). The cell paste was washed in 10⁻² M EDTA, pH 7.4, and stored at -10 C. The frozen cells were resuspended in 0.04 M triethanolamine buffer, pH 7.6, and in 0.001 M EDTA, pH 7.6, to a 15% suspension (w/v) and were disrupted in a Branson Sonifier. The temperature of the mixture was kept below 10 C during oscillation. The disrupted suspension was centrifuged in a Sorvall refrigerated centrifuge (model RC-2) at 27,000 X g for 45 min, and the supernatant fluid was assayed (extract, Table 2).

All subsequent operations were performed at 0 to 4 C. A time of 30 min was allowed to elapse between completion of ammonium sulfate additions and centrifugation.

Protamine sulfate step. The extract was adjusted to pH 7 with 0.1 N HCl and treated with a 2% solution of protamine sulfate. An amount of protamine sulfate (determined by titrating small samples of the extract) was added which precipitated large amounts of protein and nucleic acids but less than 2% of the PDR mutase activity. After 10 min at 0 to 4 C, the precipitate was removed by centrifugation, and the supernatant fluid was assayed (protamine supernatant fluid).

Ammonium sulfate fractionation. The protamine supernatant fluid was treated with solid (NH₄)₂SO₄, 167 mg/ml; the suspension was centrifuged and the pellet was discarded. Solid (NH₄)₂SO₄ (188 mg/ml) was added to the supernatant fluid; the suspension was centrifuged and the pellet was again discarded. The supernatant fluid was treated with solid (NH₄)₂SO₄ (136 mg/ml) to give 0.80 saturation; the precipitate was centrifuged, dissolved in 5 X 10⁻⁴ M phosphate buffer, pH 7.5, and dialyzed overnight against 2,000 volumes of the same buffer.

Properties of phosphodeoxyribomutase. The above procedure resulted in an apparent 10-fold purification of the enzyme; the partially purified PDR mutase, when assayed in the reaction mixture described in Table 2, showed a proportionality between enzyme activity and protein concentration. The enzyme activity had a very sharp pH optimum at pH 8; on either side of this pH, the enzyme activity fell off sharply.

Cofactor requirement. The presence of glucose-1,6-diphosphate in the reaction mixture resulted in a two- to threefold stimulation of enzyme activity. The optimal concentration for the stimulation of partially purified enzyme was 1.5 X 10⁻⁶ M (Fig. 1A). In crude extracts, less glucose-1,6-diphosphate (5 X 10⁻⁷ M) gave optimal activity (data not shown).

PDR mutase had an absolute requirement for Mn⁺⁺ (Fig. 1B). The optimal concentration of Mn⁺⁺ for the partially purified enzyme was about 4 X 10⁻⁴ M. Magnesium would not substitute for Mn⁺⁺ and had no inhibitory effect on the enzyme activity.

Sensitivity to anions and cations. PDR mutase was sensitive to low concentrations of both sulfate ion and phosphate ion; i.e., 0.03 M salt resulted in 70 to 90% inhibition of activity (Fig. 2A). The cations tested, with the exception of Mg⁺⁺, proved to be extremely inhibitory at very low concentrations (Fig. 2B). The enzyme was extremely labile. All activity was lost on freezing.

Table 2. Purification of PDR mutase from S. typhimurium

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Units/ml</th>
<th>Protein (mg/ml)</th>
<th>Specific activity</th>
<th>Total units</th>
<th>Per cent recovery</th>
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<td>34</td>
<td>75,000</td>
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<tr>
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<td>34.0</td>
<td>352</td>
<td>27,500</td>
<td>37</td>
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</table>

* The assay mixture of 1 ml contained: 40 mu moles of triethanolamine buffer, pH 7.8, 0.9 mu mole of EDTA, 0.1 mu mole of NADH, 1.0 mu mole of MnCl₂, 0.5 mu mole of glucose-1,6-diphosphate, 25 units of purified deoxyribose-5-P aldolase, 0.03 mg of alcohol dehydrogenase, and 0.9 mu mole of deoxyribose-1-phosphate. A control without deoxyribose-1-phosphate was run with each assay. This control is necessary to correct for any NADH oxidase present in the crude extracts. The level of this oxidase is usually very low and only becomes a significant correction factor when measuring low uninduced levels of the enzymes. Thus, a value of less than 0.5 unit/mg of protein is subject to a large error and is on the border line of significance. All background is removed by the protamine sulfate precipitation. One unit of PDR mutase is defined as the number of micromoles of deoxyribose-1-phosphate converted to deoxyribose-5-phosphate per hour at 25 C.
The sensitivity of this enzyme to $\text{SO}_4^{2-}$ was shown in the loss of enzyme activity in the ammonium sulfate step of the purification (Table 2). Recent data indicate, however, that the presence of Cleland's reagent (dithiothreitol) at a concentration of $10^{-4}$ M will stabilize the enzyme to some extent.

Regulation of PDR mutase: induction in the wild-type strain by 2-deoxyribose. When deoxyribose was added (final concentration, 0.2%) to a growing culture of *S. typhimurium* (Klett optical density at 75 units), there was an increase in the levels of deoxyribose kinase, deoxyribose-5-P aldolase, PDR mutase and thymidine phosphorylase (Fig. 3). Fructose diphosphate aldolase, which served as a control enzyme, did not change in specific activity during the induction of the other four enzymes. The induction curve for

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**FIG. 1.** Effect of cofactors on PDR mutase activity. (A) PDR mutase was assayed as described in Table 2 with the specified amount of glucose-1,6-diphosphate added. (B) PDR mutase was assayed as described in Table 2, but without EDTA and in the presence of various amounts of $\text{Mn}^{2+}$. Values on the ordinate are given as units of enzyme per microliter of a partially purified preparation with a specific activity under optimal conditions of $\text{Mn}^{2+}$ and glucose-1,6-diphosphate of 350 units/mg of protein.

**FIG. 2.** Effect of anions and cations on PDR mutase activity. PDR mutase was assayed as in Table 2, but without EDTA and in the presence of $1.5 \times 10^{-4}$ M glucose-1,6-diphosphate and $4 \times 10^{-4}$ M $\text{Mn}^{2+}$. An activity of 100% corresponds to a preparation with a specific activity of 350 units/mg of protein.
deoxyribose-5-P aldolase appeared to be diphasic. As a result, this enzyme reached maximal levels of induction about 1 hr after the other enzymes. The second phase of deoxyribose-5-P aldolase induction from 60 to 100 min did not seem to be coordinate with the other three enzymes, suggesting that this enzyme is under a different regulation than deoxyribose kinase, PDR mutase, and thymidine phosphorylase.

**Induction of drbCl by thymidine.** The isolation of drbCl, a deoxyribose-negative mutant, was previously described (12). This mutant remained uninduced for all of the enzymes, i.e., deoxyribose-5-P aldolase, PDR mutase, deoxyribose kinase, and thymidine phosphorylase, when grown in the presence of deoxyribose (Table 3). It had low uninduced levels of all of the enzymes and was presumed to be a regulatory mutant in the deoxyribose (drb) operon. When this mutant was grown in the presence of thymidine (Fig. 4), coordinate induction of PDR mutase, thymidine phosphorylase, and deoxyribose-5-P aldolase was observed. Once again, fructose diphosphate aldolase did not change in specific activity. In contrast to the wild-type induction curve (Fig. 3), the deoxyribose-5-P aldolase induction curve was no longer diphasic, the specific activity of deoxyribose-5-P aldolase was about two-thirds of the wild-type level when grown on deoxyribose, and levels of maximal induction of all three enzymes were obtained about the same time. Also, when this mutant was grown on thymidine, there was no induction of deoxyribose kinase; this shows that deoxyribose kinase is regulated independently from the PDR mutase, thymidine phosphorylase, and deoxyribose-5-P aldolase.

**Induction of deoC4001 in the presence and absence of deoxyribose.** Mutant deoC4001 is a thymine-requiring mutant isolated as a secondary low thymine requirer (12). When deoC4001 was grown in Casamino Acids medium containing thymine (10 µg/ml) but without any inducer, there was initially (zero time, Fig. 5A) four to five times the level of PDR mutase and thymidine phosphorylase seen in wild-type uninduced cells (Fig. 3), but there was no change in levels of deoxyribose-5-P aldolase or deoxyribose kinase. These high levels of PDR mutase and thymidine phosphorylase increased further during growth. Why this sharp increase in levels of these enzymes occurred during growth is not clear, but presumably it could be due to an increase in the internal accumulation of deoxyribose-5-phosphate. When deoxyribose was added to the growing culture (Fig. 5B), induction was observed of both deoxyribose kinase and deoxyribose-5-P aldolase, presumed in this case to be deoxyribose-5-P aldolase type I because of its late induction and because it is not regulated with PDR mutase and thymidine phosphorylase. Levels of PDR mutase and thymidine phosphorylase also increased, and at a faster
Table 3. Enzyme levels in the wild type and various mutant strains of Salmonella typhimurium

<table>
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<tr>
<th>Strain</th>
<th>Addition to media</th>
<th>Units/mg of protein</th>
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<tr>
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<td>DRSP aldolase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DR kinase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PDR mutase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Thymidine phosphorylase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>FDP aldolase&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> The strains were grown and induced as described in Materials and Methods. Additions were at the following final concentrations: deoxyribose, 0.2%; thymidine, 0.05%.

<sup>b</sup> Alumina-ground extracts were assayed spectrophotometrically for deoxyribose kinase (DR kinase), deoxyribose-5-P aldolase (DRSP aldolase), PDR mutase, and fructose diphosphate aldolase (FDP aldolase). The reaction mixtures of 1 ml contained, for deoxyribose kinase: 28 μmoles of triethanolamine buffer, pH 7.6, containing 10<sup>-4</sup> M EDTA, 0.1 μmole of NADH, 10 μmoles of MgCl₂, 1 μmole of NaF, 10 μmoles of adenosine triphosphate, 25 units of crystalline deoxyribose-5-P aldolase, 0.01 mg of α-glycerol phosphate dehydrogenase triose phosphate isomerase, and 10 μmoles of deoxyribose-5-P aldolase. A control without deoxyribose was run with each assay (see footnote of Table 2). The reaction mixture for deoxyribose-5-P aldolase contained, in 1 ml: 36 μmoles of triethanolamine buffer, pH 7.6, containing 10<sup>-4</sup> M EDTA; 0.1 μmole of NADH; 0.03 mg of alcohol dehydrogenase; and 0.5 μmole of deoxyribose-5-P-phosphate. A control without deoxyribose-5-P-phosphate was run with each assay. One unit of deoxyribose-5-P aldolase is defined as the number of micromoles of deoxyribose-5-phosphate split per hour at 25°C. PDR mutase was assayed as described in Table 1. The reaction mixture for FDP aldolase contained in 1 ml: 40 μmoles of triethanolamine buffer, pH 7.6; 0.1 μmole of NADH; 1 μmole of KCl; 0.01 mg of α-glycerol phosphate dehydrogenase triose phosphate isomerase; and 2 μmoles of fructose-1,6-diphosphate. A control without fructose diphosphate was run with each assay. One unit of FDP aldolase is defined as the number of micromoles of fructose diphosphate times 2 split per hour at 25°C.

<sup>c</sup> Thymidine phosphorylase was assayed as described in Materials and Methods. The reaction mixture of 0.2 ml contained 3.7 μmoles of thymidine, 40 μmoles of tris(hydroxymethyl)aminomethane chloride buffer, pH 7.4, and 20 μmoles of Na arsenate. The reaction was run at 37°C, and samples were removed at 0, 5, and 10 min; 1 ml of 0.3 M NaOH was added to stop the reaction. The increase in optical density was measured at 300 nm. One unit is defined as the number of micromoles of thymine found per hour at 37°C. An extinction coefficient of 4.04 was used.

<sup>d</sup> Not assayed.

Rate than in the absence of deoxyribose. These data suggest the presence of separate operons in S. typhimurium, a deo operon consisting of PDR mutase, thymidine phosphorylase, and deoxyribose-5-P aldolase, type II (missing in deoC4001), and the drb operon consisting of deoxyribose kinase. It is not clear whether deoxyribose-5-P aldolase, type I, is also induced by deoxyribose or by a product of deoxyribose metabolism, or how it is regulated with respect to the above two operons. From the induction curve in the wild-type organism (Fig. 3), it would seem that deoxyribose-5-P aldolase, type II, is induced early, along with deoxyribose kinase, PDR
mutase, and thymidine phosphorylase, but that type I is induced much later than all of the other enzymes. Similarly, deoxyribose-5-P aldolase, type I, in deoC4001 appears later than the other enzymes and only after significant levels of deoxyribose kinase have been induced.

Deoxyribose-5-phosphate as the inducer of the deo operon. Three lines of evidence point to the role of deoxyribose-5-phosphate as the inducer of the deo operon. (i) Table 3 and Fig. 5 show that mutants lacking or deficient in deoxyribose-5-P aldolase (deoC4006, deoC4008) became pheno-
typically constitutive for both thymidine phosphorylase and PDR mutase. These mutants during normal growth apparently accumulate deoxy-
ribose-5-phosphate which acts as an endogenous inducer of the enzymes. (ii) Mutants which are deficient in PDR mutase, i.e., deoB4012, are fully induced by growth on deoxyribose, where the phosphate ester is produced via deoxyribose kinase, but on thymidine these mutants are poorly induced for both deoxyribose-5-P aldolase and thymidine phosphorylase. These mutants are un-
able to break down thymidine to deoxyribose-5-phosphate and thus remain poorly induced. (iii) Mutants which remain uninduced for deoxyribose kinase by deoxyribose (i.e., drbCl) also remain

**Fig. 4.** Kinetics of enzyme induction in mutant drbCl by thymidine. The experiment was performed as described in legend of Fig. 3 with the exception that thymidine (final concentration, 0.05%) was added in place of 2-deoxyribose to a log-phase culture (Klett optical density of 75). Deoxyribose kinase activity (▲), deoxyribose-5-P aldolase activity (●), PDR mutase activity (X), thymidine phosphorylase activity (Δ), and FDP aldolase activity (○).

**Fig. 5.** Kinetics of enzyme induction in mutant deoC4001 in the presence and absence of deoxyribose. The experiment was performed as described in legend of Fig. 3 except that no additions were made to the culture shown on the left and the cells were grown in Casamino Acids containing thymine (10 μg/ml). To the right-hand culture, deoxyribose (final concentration, 0.2%) was added at zero time at a Klett optical density of 75. Samples were removed from both cultures at the same time intervals. Zero time for both cultures is the time deoxyribose is added to the culture shown on the right. Deoxyribose kinase activity (X), deoxyribose-5-P aldolase activity (○), PDR mutase activity (●), thymidine phosphorylase activity (□), and FDP aldolase activity (△).
uninduced for deoxyribose-5-P aldolase, PDR mutase, and thymidine phosphorylase. They can, however, be induced by thymidine for the enzymes in the deo operon (Fig. 4).

Genetic studies on the deo operon of S. typhimurium. Interrupted-mating experiments with the donor strain, Hfr A, and a recipient which is PDR mutase-negative, threonine-negative, were performed as shown in Fig. 6. The threonine marker entered the recipient at about 19.5 min and the PDR mutase marker about the same time. Attempts to show cotransduction between PDR mutase and threonine, or deoxyribose-5-P aldolase and threonine, have revealed no linkage when PLT22 was used as the transducing phage. Transduction studies have shown, however (see Table 4), that the structural genes for PDR mutase, deoxyribose-5-P aldolase, type II, and thymidine phosphorylase are linked together and transduced on the same transducing fragment. The structural gene for deoxyribose-5-P aldolase, type II, shows an 80 to 100% linkage to the structural gene for thymidine phosphorylase, depending on the particular phosphorylase-negative mutant used. The deoxyribose-5-P aldolase, type II, structural gene also shows an 88 to 95% linkage to the structural gene for PDR mutase. The PDR mutase structural gene, although closely linked to the gene for deoxyribose-5-P aldolase, type II, shows only a 27 to 56% linkage to the thymidine phosphorylase gene. In these transduction studies, selection was made for deoxyribose-5-P aldolase, type II, by selecting on Endo-deoxyribose agar. The high concentration of deoxyribose in this medium (0.8%) prevents the growth of mutants deficient in deoxyribose-5-P aldolase; positive colonies on these plates have received the deoxyribose-5-P aldolase marker from the donor. When deoC4006, a mutant negative for both deoxyribose-5-P aldolase type I and type II, was used, the positive colonies appeared to have received only type II deoxyribose-5-P aldolase. This was shown by selecting a deoxyribose-5-P aldolase-positive transductant which was deficient in PDR mutase (deoC4006-T), and testing the enzyme levels in this strain (Table 3). The level of deoxyribose 5-P-aldolase in deoC4006-T which has been transduced to PDR mutase-negative, Endo-deoxyribose positive is only about 70% of that of the wild type, but it is equivalent to the level of deoxyribose-5-P aldolase found in drbCl grown on thymidine, where only type II is induced. Also, deoC4006-T is no longer constitutive for thymidine phosphorylase but is inducible by deoxyribose.

**DISCUSSION**

The regulation of the 2-deoxyribose gene-enzyme complex in *S. typhimurium* appears to be unique in that one operon controls the induction of a second operon via an intermediate of the first biosynthetic pathway. Evidence presented in this report suggests that the enzymes thymidine phosphorylase, deoxyribose-5-P aldolase, type II, and PDR mutase are controlled by a sequence of linked genes which map close to the threonine locus in *S. typhimurium.* The inducer of this pathway, called the deo operon by Lomax and Greenberg (16), is deoxyribose-5-phosphate, a product of both thymidine and deoxyribose metabolism in *S. typhimurium.* The structural gene for deoxyribose kinase appears to be unlinked to the deo operon (12). Deoxyribose kinase appears to be induced by 2-deoxyribose, but the inducer of deoxyribose-5-P aldolase, type I, is not yet known. Although it originally seemed that this type I deoxyribose-5-P aldolase was regulated with deoxyribose kinase and induced by 2-deoxyribose (12), the kinetic data presented in this paper are not consistent with this hypothesis. The second phase of deoxyribose-5-P aldolase induction does not seem to be coordinate with any of the other enzymes in the complex, being induced much later and seeming to depend on the induction of significant levels of deoxyribose kinase.
Our data suggest that deoxyribose-5-phosphate may also be the inducer of deoxyribose-5-P aldolase, type I, but only when present at much higher concentrations than needed to induce deoxyribose-5-P aldolase, type II, and the rest of the deo operon. Further studies are in progress to clarify the regulation of the deoxyribose-5-P aldolase, type I, and its relation to deoxyribose kinase.

The two pathways, the deoxyribose pathway and the deoxyribonucleoside pathway, are interconnected metabolically by the presence of the enzyme PDR mutase. This enzyme has proved somewhat difficult to handle in vitro because of its extreme sensitivity to both cations and anions. Sensitivity to SO₄²⁻ and PO₄³⁻ has also been reported for this enzyme from E. coli (14). It has an absolute requirement for Mn⁺⁺ which is not satisfied by magnesium ion. This is perhaps one of the few cases known where a manganese-requiring enzyme will not give at least some activity with magnesium ion. The cofactor, glucose-1,6-diphosphate, noted as an enhancer of PDR mutase by previous workers (24), also serves as a stimulating factor for the enzyme isolated from S. typhimurium. It may, however, not be the true cofactor in vivo. A more likely compound is deoxyribose-1, 5-diphosphate. Further characterization of the properties of this enzyme and the role of a diphosphate awaits its more extensive purification.

A clarification of the various types of mutants involved in this gene-enzyme complex has been facilitated by the recent work with E. coli on thymine-requiring mutants (1, 2, 4, 5, 10, 15, 16, 19). It seems clear now that in both S. typhimurium and E. coli the low thymine-requiring mutants are those which are deficient in either deoxyribose-5-P aldolase or phosphodeoxyribo- mutase; this metabolic defect could eventually lead to an increase in the available pool of deoxyribose-1-phosphate, a necessary substrate for the conversion of thymine to thymidine (4). Mutants deficient in thymidine phosphorylase, PDR mutase, or deoxyribose-5-P aldolase, type II, can easily be isolated by using the thymine requirement as a selection technique. Selection of low thymine-requiring mutants from high thymine-requiring mutants (those deficient only in thymidylate synthetase) resulted in selection of mutants deficient either in PDR mutase or in deoxyribose-5-P aldolase. The deoxyribose-5-P aldolase mutants are characterized by an inhibition of growth in the presence of deoxyribose and
thymidine. An extensive study of this growth inhibition has recently been reported (3). Growth of PDR mutant-deficient mutants, on the other hand, does not appear to be sensitive to either of those compounds at the concentrations tested.

Another important characteristic of deoxyribose-5-P aldolase-negative mutants is that they become phenotypically constitutive for both thymidine phosphorylase and PDR mutase. Deoxyribose kinase and deoxyribose-5-P aldolase, type I, on the other hand, are induced only when the cells are grown in the presence of deoxyribose, as shown with deoC4001. PDR mutant mutants do not become phenotypically constitutive and are poorly inducible by thymidine, but when grown on 2-deoxyribose they are fully inducible for deoxyribose kinase, thymidine phosphorylase, and deoxyribose-5-P aldolase. All of these data point to deoxyribose-5-phosphate as the inducer of the deo operon, as previously suggested (2, 4, 5, 12, 16).

A comparison of the work done in E. coli and S. typhimurium suggests that the deo operon is quite similar in the two organisms; the deo A, deo B, and deo C genes of Lomax and Greenberg (16) correspond to the thymidine phosphorylase, PDR mutase, and deoxyribose-5-P aldolase, type II, genes of S. typhimurium. The order of the genes is as yet not certain. In both organisms, the deo operon is close to threonine on the chromosome. However, in E. coli, the deo operon is cotransducible with threonine, whereas in S. typhimurium it is not, probably owing to the smaller transducing fragment carried by PLT22.

Work is now in progress to order the structural genes in the deo operon of S. typhimurium and to determine whether this operon is under positive or negative control.

ACKNOWLEDGMENTS

We thank Lydia Pantoja for excellent technical assistance in many phases of this investigation. We are grateful to M. Lomax and R. Greenberg for sending us their manuscript prior to publication.

This investigation was supported by Public Health Service grants FR-05416-05 and 1 ROI AM 12152-01 from the National Institute of Arthritis and Metabolic Diseases.

LITERATURE CITED