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2-Deoxyribose Gene-Enzyme Complex in *Salmonella typhimurium*

I. Isolation and Enzymatic Characterization of 2-Deoxyribose-Negative Mutants[1]

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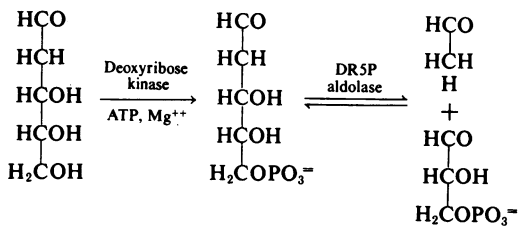
Salmonella typhimurium was found to utilize 2-deoxyribose as a sole carbon and energy source. Cells grown in the presence of deoxyribose contained increased levels of deoxyribose kinase, thymidine phosphorylase, and two forms of deoxyribose-5-phosphate aldolase (DR5P aldolase). One form of DR5P aldolase was induced by deoxyribose and coordinately regulated with deoxyribose kinase. The second form of DR5P aldolase was induced by deoxyribose-5-phosphate and coordinately regulated with thymidine phosphorylase. Mutants unable to ferment deoxyribose have been isolated and shown to be lacking either deoxyribose kinase or deoxyribose permease, but none has been found from which DR5P aldolase is missing. Thymine-requiring mutants which are able to grow on low levels of thymine have been isolated and shown, in some cases, to be lacking one or both DR5P aldolases.

2-Deoxy-D-ribose was shown by Domagk and Horecker (3) and by Ginsberg (4) to be fermented by *Lactobacillus plantarum* by the following inducible pathway:



The first step in the fermentation is the phosphorylation of deoxyribose, catalyzed by deoxyribose kinase (DR kinase) in the presence of adenosine triphosphate (ATP) and magnesium ion (4). The second step is the reversible cleavage of the phosphate ester to acetaldehyde and D-glyceraldehyde-3-phosphate, catalyzed by 2-deoxyribose-5-phosphate aldolase (DR5P aldolase). Subsequently, DR5P aldolase was purified (10) and crystallized (6) from this organism.

Earlier, Racker (11) had described a similar DR5P aldolase present in extracts of *Escherichia coli* and *Cornyebacterium diphtheria*, and in mammalian liver. He reported that *E. coli* was unable to grow on 2-deoxyribose as a sole carbon and energy source, although another paper reported that some strains of *E. coli* can adapt to grow on 2-deoxyribose (7).

Recently, we have found that *Salmonella typhimurium* LT2 will utilize 2-deoxyribose as a sole carbon and energy source. This report describes the induction of the enzymes in *S. typhimurium* necessary for the fermentation of deoxyribose and the isolation and characterization of mutants of *S. typhimurium* unable to grow on 2-deoxyribose.

MATERIALS AND METHODS

Bacterial strains. S. typhimurium LT2 (C^+dg^s) and a glucose-resistant mutant of this strain (C^+dg^{r-1}) were obtained from E. Englesberg. *S. typhimurium* strain Q-1 was obtained from M. Watanabe. All of the deoxyribose-negative mutants were derived from these strains. The cultures were maintained on nutrient agar slants. An HfrA strain described by Zinder and an $HfrB_3$ strain described by Sanderson (12) were obtained from D. Kessler.

Materials. Commercial preparations were used unless otherwise specified. 2-Deoxyribose was ob-

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tained from International Chemical and Nuclear Corp., City of Industry, Calif. 2-Deoxyribose-5-phosphate was synthesized with purified DR5P aldolase according to the procedure of Pricer and Horecker (10), or was purchased from Calbiochem, Los Angeles, Calif. Alcohol dehydrogenase and glycerol phosphate dehydrogenase-triose phosphate isomerase mixture 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. (13).

Culture and growth of organisms. For the various experiments cells were grown in Casamino Acids medium buffered with phosphate at *p*H 7.0, in Nutrient Broth (Difco), or in a synthetic medium of the following composition (per cent, w/v): KH_2PO_4, 0.3; K_2HPO_4, 0.7; $(NH_4)_2SO_4$, 0.1; $MgSO_4$, 0.01; and carbon sources as indicated. The carbon sources and salts were sterilized separately and added as concentrated solutions. Solid medium has 15 g of agar added per liter. Endo-deoxyribose agar is standard Endo agar (Difco) with 0.8% deoxyribose in place of lactose.

Induction of enzymes. Induction of the wild-type organism was accomplished by adding deoxyribose to the cells during logarithmic growth in Casamino Acids medium and harvesting the cells when the inducer was 90% utilized (about 1 hr at a final concentration of deoxyribose of 0.1%). The deoxyribose-negative mutants were grown to log phase in Casamino Acids medium, and inducer (deoxyribose; final concentration, 0.1%) was added 4 hr prior to harvesting. The cells were washed in 10^{-3} M ethylenediaminetetraacetate (EDTA) and stored frozen at −10 C.

Preparation of extracts. Extracts of the cells were made by grinding the frozen pellets with alumina (twice the wet weight). The mixture was suspended in 10^{-3} M EDTA plus 10^{-2} M mercaptoethanol and then centrifuged for 30 min at 27,000 × *g*. The supernatant fluids were then assayed for enzyme activities and protein concentration.

Enzyme assays. DR5P aldolase was assayed spectrophotometrically by measuring the conversion of acetaldehyde to ethyl alcohol in the presence of 2-deoxyribose-5-phosphate, reduced nicotinamide adenine dinucleotide ($NADH_2$), and alcohol dehydrogenase (11). DR kinase was measured in a coupled assay system containing 2-deoxyribose, ATP, magnesium ion, DR5P aldolase, $NADH_2$, glycerol phosphate dehydrogenase, and triose phosphate isomerase. The assay determines the amount of 2-deoxyribose phosphorylated in the kinase reaction by first converting the phosphate ester to glyceraldehyde-3-phosphate in the presence of excess crystalline DR5P aldolase. The glyceraldehyde-3-phosphate is then converted to glycerol phosphate in the presence of triose phosphate isomerase and glycerol phosphate dehydrogenase. The latter reaction requires the oxidation of $NADH_2$ to NAD, enabling us to follow the complete sequence of reactions spectrophotometrically at 340 mμ in a Beckman recording spectrophotometer equipped with a Gilford automatic recorder.

Thymidine phosphorylase was assayed by measuring the formation of thymine from thymidine in the presence of arsenate (Breitman, *personal communication*). Thymine was measured in alkaline solution at 300 mμ.

Protein determination. Protein was determined by the method of Lowry et al. (8) or that of Bücher (2) with fructose diphosphate aldolase as a standard.

Isolation of deoxyribose-negative mutants. A wild-type culture of *S. typhimurium* was grown to log phase in Nutrient Broth. Ethylmethane sulfonate (0.03 ml) was added to 1-ml samples of the culture, and the samples were incubated for 30 min at 37 C without shaking. The cells were centrifuged, washed twice in cold saline, and then diluted into 0.2% minimal glucose medium. The minimal glucose tubes were incubated with shaking overnight at 37 C. The cultures were then plated on Endo-deoxyribose agar plates or minimal glucose plates. Pink colonies were picked directly from the Endo-deoxyribose plates, restreaked, purified, and cultured on agar slants. The colonies from the minimal glucose plates were replicated to minimal glucose and minimal deoxyribose plates. Those colonies growing on the former and not on the latter were restreaked, purified, and cultured on agar slants. Mutants unable to ferment deoxyribose are denoted as *dxr*, followed by the number of isolation (i.e., dxr-1, dxr-2, etc.). The method of replica plating was employed to avoid selecting against mutants which might be inhibited by high concentrations of deoxyribose.

Isolation of thymine-requiring mutants. Thymine-requiring mutants were isolated by the aminopterin method (9). Wild-type *S. typhimurium* was grown to stationary phase in minimal glucose media. A loopful of this culture was inoculated into 1 ml of minimal glucose selection medium containing 600 μg of aminopterin, 200 μg of thymine or thymidine, and 3 μg of nitrosoguanidine. The culture was incubated at 37 C with shaking until turbidity appeared (2 to 3 days). The cultures were plated on minimal glucose plates containing 20 μg of thymine per ml. The colonies were then replicated to minimal glucose plates and minimal glucose plus thymine plates. Those mutants which required thymine were picked, retested for purity, and cultured on agar slants. Secondary mutants able to grow on low levels of thymine (2 μg/ml) were isolated by the method of Harrison (5).

Interrupted matings. Interrupted matings were performed as described by Sanderson (12). The donor strains were Hfr A and Hfr B_3. Overnight broth cultures were diluted 1:40 in broth and grown to mid-log phase with gentle shaking at 37 C. About 2×10^8 donor cells and 10^9 recipient cells were mixed on a membrane filter (25-mm diameter, 0.45-μ 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 then transferred to 16 ml of nutrient broth (zero-time) at 37 C, and the mating was continued with gentle agitation. At various time intervals, samples of the mating mixture were diluted in broth and the mated pairs were separated by shaking for 2 min on a Vortex Junior Mixer. Samples were pipetted into 2 ml of soft minimal deoxyribose agar and poured on

minimal deoxyribose plates. Growth of the donor cells was prevented by omitting from the minimal medium the supplements required by the auxotrophic donor strains.

RESULTS

Growth on 2-deoxyribose. Figure 1 illustrates the growth of *S. typhimurium* on minimal deoxyribose medium. Deoxyribose allowed about one-third the growth obtained from an equal amount of glucose, presumably because only the glyceraldehyde-3-phosphate portion of the molecule is utilized for energy (3). A typical diauxic growth curve was observed when the cells were grown in a glucose plus deoxyribose medium, indicating that the deoxyribose fermentation pathway is an inducible one.

Induction of DR kinase and DR5P aldolase by deoxyribose. When deoxyribose was added (final concentration, 0.1%) to a growing culture of *S. typhimurium*, there was an increase in the levels of DR kinase and DR5P aldolase, as shown in Fig. 2. The enzymes appeared to be induced coordinately for a period of 75 min, after which time the inducer was used up from the media and the specific activity of both enzymes decreased.

When the concentration of deoxyribose was increased from 0.1 to 0.5% (shown in Fig. 3), a somewhat different pattern of induction was observed. The specific activities of DR kinase and DR5P aldolase were still coordinated during the first hour. However, if deoxyribose was present in the media after the first hour, the specific activity of DR5P aldolase continued to increase, while that of DR kinase remained constant.

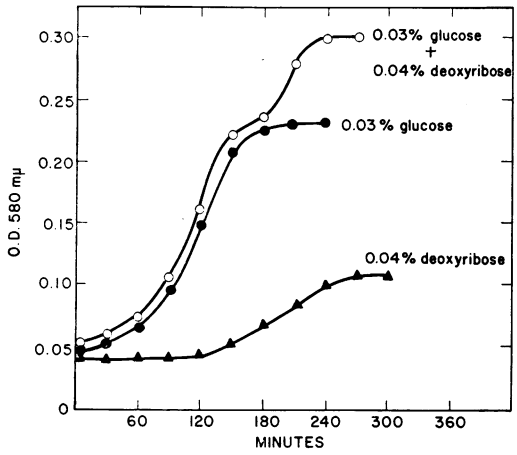

FIG. 1. *Growth of Salmonella typhimurium on minimal glucose and minimal deoxyribose medium. Overnight cultures were diluted into fresh minimal glucose medium and grown to log phase. The cells were centrifuged, washed with cold saline, and resuspended to an optical density of 0.8. At zero-time, 0.02 ml of the culture was added to 0.03% glucose (●), 0.04% deoxyribose (▲), and 0.03% glucose + 0.04% deoxyribose medium (○), and the increase in optical density was followed. Cultures with no carbon source (not shown) showed no increase in optical density but a slight decrease was observed after 3 hr.*

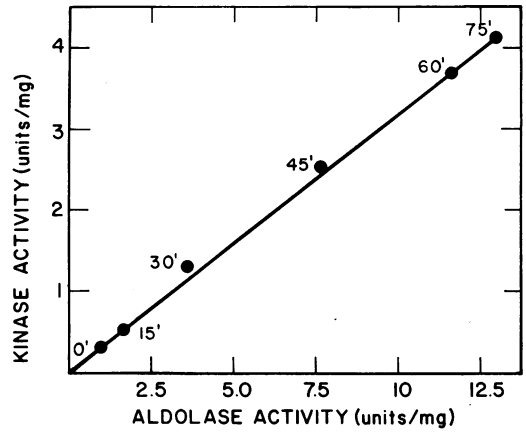

FIG. 2. *Induction of DR kinase and DR5P aldolase in Salmonella typhimurium by deoxyribose. Cells were grown to log phase in Casamino Acids medium. At zero-time, 2-deoxyribose (final concentration = 0.1%) was added to the growing culture, and samples of cells were removed at the times indicated. The samples were centrifuged, washed with 10^{-3} M EDTA, and kept frozen. The frozen pellets were extracted by alumina grinding, and the extracts were assayed for DR kinase activity, DR5P aldolase activity, and protein, as described in Materials and Methods and in Table 1.*

A more detailed study of the kinetics is shown in Fig. 4, where induction of the two enzymes has been plotted against time after addition of inducer (the same picture was seen when the data were plotted against increase in cell mass). After the first hour of induction, there was a shut-off and then an increase in the specific activity of DR5P aldolase, which resulted in a diphasic induction curve. The specific activity of DR kinase, on the other hand, increased initially and then remained unchanged after 45 min. After 120 min, the inducer had been used up from the media, resulting in a decrease in the specific activity of both DR kinase and DR5P aldolase.

Isolation and enzymatic characterization of deoxyribose-negative mutants. Mutants unable to ferment deoxyribose have been isolated by the procedure described in Materials and Methods. Each mutant was grown in Casamino Acids medium + 0.1% deoxyribose for 4 hr and then harvested. Alumina-ground extracts were assayed for DR kinase and DR5P aldolase activity.

The mutants isolated fell into three main groups

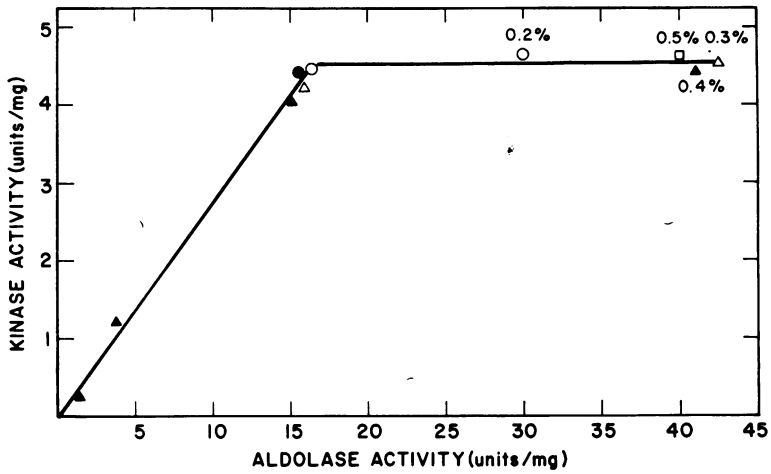

FIG. 3. *Effect of increasing deoxyribose concentration on the induction of DR kinase and DR5P aldolase. Cells were grown to log phase in Casamino Acids medium. Deoxyribose was added to five flasks at final concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5%. After 1 hr, a sample of cells was removed from each flask and cell-free extracts were assayed for DR kinase, DR5P aldolase, and protein. After 2, 3, 4, and 5 hr, samples were removed from the flasks containing 0.2% (○), 0.3% (△), 0.4% (▲), and 0.5% (□), final concentration, of deoxyribose, respectively. In addition, samples were taken from the flask containing 0.4% at zero-time and at 30 min. Cells sampled at each point were extracted by alumina grinding, and the extracts were assayed for DR kinase, DR5P aldolase, and protein as described in Materials and Methods and in Table 1.*

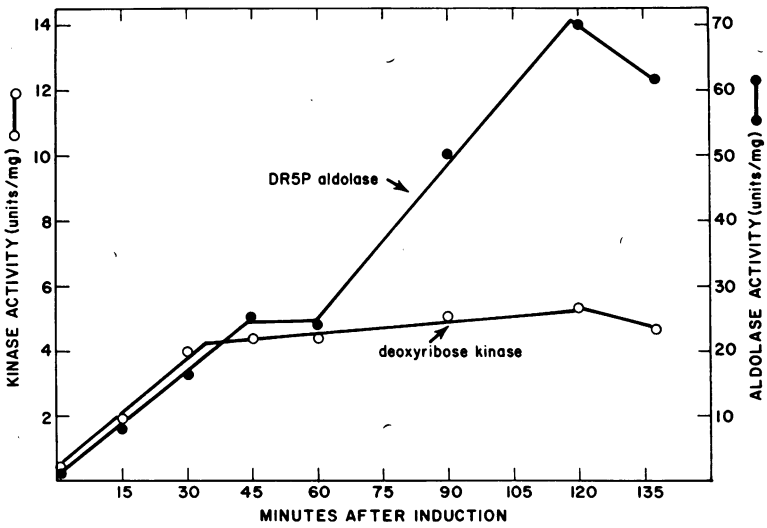

FIG. 4. *Induction of DR kinase and DR5P aldolase by 0.2% deoxyribose. Cells were grown in Casamino acids medium to log phase. At zero-time, deoxyribose (final concentration = 0.2%) was added to the culture, and samples of cells were removed at the times indicated. Cells sampled at each point were extracted by alumina grinding, and the extracts were assayed for DR kinase, DR5P aldolase, and protein. Deoxyribose was used up from the medium at 135 min.*

(Table 1). Mutants in the first group had no detectable or very low levels of induced DR kinase as compared to the wild type-induced levels. In addition, some of these mutants had lower levels of induced DR5P aldolase than that found in the wild-type strains. The mutants in this group have been tentatively classified as DR kinase-negative mutants. Mutants in the second group had nearly normal levels of induced DR kinase and DR5P aldolase as compared to the wild-type levels.

TABLE 1. *Enzyme levels in wild-type strains and dxr-negative mutants*

Group	Strain	Deoxyribose kinase[a] (units/mg)		DR5P aldolase[a] (units/mg)	
		Uninduced	Induced	Uninduced	Induced
(WT)	C^+dg^s	0.8	4.0	1.4	24
	C^+dg^{r-1}	0.3	3.7	1.0	33
	Q-1	0.7	3.7	0.8	30
	Q-1 gal-1	1.3	3.7	3.2	32
Kinase-negative	dxr-23		<0.02		3.3
	dxr-37a		<0.02		2.4
	dxr-50d		<0.02	1.1	6.1
	dxr-22a		0.2		5.3
	dxr-39a		0.2		19.0
	dxr-48b		0.4	1.0	12.0
	dxr-55		0.8		23.5
	dxr-34		0.8	0.9	24.0
	dxr-44a		0.9	1.0	30.4
Transport-negative	dxr-36a		3.0		28.0
	dxr-41c		1.7		28.0
	dxr-42a		1.9		18.5
	dxr-53		1.8		23.0
	dxr-66		3.2		25.0
	dxr-69		2.1		25.0
Regulatory or polar	dxr-1		0.2		1.0
	dxr-26		0.5		1.0
	dxr-4		0.1		2.0
	dxr-5		0.03		1.4
	dxr-6		0.3		2.1
	dxr-12a		<0.02		1.8
	dxr-14a		<0.02		1.6
	dxr-24		0.4		1.6
	dxr-38		0.4		1.4
	dxr-40b		0.1		2.3
	dxr-52		0.2		2.9
	dxr-54		0.3		2.5
	dxr-60		0.6		2.6
	dxr-62		0.5		0.7

[a] Deoxyribose-negative mutants were grown and induced as described in Materials and Methods. Alumina-ground extracts were assayed spectrophotometrically for DR kinase and DR5P aldolase. The reaction mixtures of 1 ml contained, for DR kinase: 28 μmoles of triethanolamine buffer, *p*H 7.6, containing 10^{-3} M EDTA; 0.1 μmole of $NADH_2$; 10 μmoles of $MgCl_2$; 10 μmoles of NaF; 10 μmoles of ATP; 25 units of crystalline DR5P aldolase; 0.01 mg of α-glycerol phosphate dehydrogenase/triose phosphate isomerase, and 10 μmoles of deoxyribose. A control without deoxyribose was run with each assay. The reaction mixture for DR5P aldolase contained, in one ml: 36 μmoles of TEA buffer, *p*H 7.6, containing 10^{-3} M EDTA; 0.1 μmole of $NADH_2$; 0.03 mg of alcohol dehydrogenase, and 5 μmoles of deoxyribose-5-phosphate. A control without deoxyribose-5-phosphate was run with each assay. One unit of kinase is defined as the number of micromoles of deoxyribose phosphorylated per hour at 25 C. One unit of aldolase is defined as the number of micromoles of deoxyribose-5-phosphate split per hour at 25 C.

These mutants take up deoxyribose from the medium at a reduced rate (~one-tenth the wild-type rate). They have been classified as transport-negative mutants. The mutants which fell in the third group were the type of mutant most often found. They had low but detectable levels of DR kinase and DR5P aldolase. The levels of enzymes in these mutants after induction were equivalent to the uninduced levels found in the wild-type strains. These mutants have been classified as regulatory or polar mutants.

The fourth group of mutants to be expected, namely, DR5P aldolase-negative with active DR kinase, have not as yet been detected in over 100 mutants isolated directly as *mutants unable to ferment deoxyribose*.

Isolation and characterization of thymine-requiring mutants. A recent paper by Breitman and Bradford (1) reported that a low thymine-requiring mutant of *E. coli* was DR5P aldolase-negative. An attempt was made, therefore, to isolate DR5P aldolase-negative mutants of *S. typhimurium* by selection of thymine-requiring strains in *Salmonella*. Secondary mutants were isolated from thymine-requiring mutants which grow on 20 μg/ml of thymine by the method of Harrison (5). These secondary mutants required only 2 μg/ml of thymine per ml for growth and fell into four groups with respect to levels of DR5P aldolase activity (Table 2). Mutants in the first group, represented by Thy_2-1, fermented deoxyribose and contained normal induced levels of DR kinase, DR5P aldolase, and thymidine phosphorylase. Mutants in the second group, represented by Thy_2-6 did not ferment deoxyribose, and their growth was inhibited in the presence of high concentrations of deoxyribose. These mutants had nearly normal levels of induced DR kinase but were negative for DR5P aldolase, and appeared partially constitutive for thymidine phosphorylase. Mutants in the third group, represented by Thy_2-21, did not ferment deoxyribose and their growth was inhibited by 0.8% deoxyribose. These mutants had normal inducible levels of DR kinase, but appeared "constitutive" for DR5P aldolase and thymidine phosphorylase. Mutants in the fourth group, represented by Thy_2-42, fermented deoxyribose but were inhibited in the presence of high concentrations (0.8%) of deoxyribose. They con-

tained normal induced DR kinase and low but *inducible* levels of DR5P aldolase (~one-half the level of the wild type). Thymidine phosphorylase in these mutants appeared "constitutive." These results are summarized in Table 3, and are interpreted later according to the model given in the Discussion.

Induction by deoxyribose-5-phosphate. Since indirect evidence indicated that deoxyribose-5-phosphate might be the inducer of the second form of DR5P aldolase, the following experiment was performed. Mutant dxr-1, a regulatory mutant (see Table 1), remained uninduced for DR kinase, DR5P aldolase, and thymidine phosphorylase when grown in the presence of deoxyribose. Growth of this mutant, however, in the presence of deoxyribose-5-phosphate (Table 4), resulted in induction of DR5P aldolase and thymidine phosphorylase but not DR kinase. The level of DR5P aldolase was about one-half that found in the wild-type strain grown in the presence of deoxyribose, whereas the thymidine phosphorylase level was about the same. It was difficult to interpret the induction in the wild-type strain grown in the presence of deoxyribose-5-phosphate, since any breakdown of deoxyribose-5-phosphate to deoxyribose would result in induction of DR kinase, DR5P aldolase, and thymidine phosphorylase. The fact that there was some induction of DR kinase in the wild type by dexoyribose-5-phosphate would indicate that some breakdown did occur.

TABLE 2. *Characterization of low thymine-requiring strains of Salmonella typhimurium*

Mutant[a]	DR5P aldolase[b]	Deoxyribose kinase[b]	Thymidine phosphorylase[c]
	units/mg	*units/mg*	*units/mg*
Thy_2-1			
(1) CA medium.....	1.5	0.1	13.8
(2) CA + 0.2% DR.	20.0	5.3	69.0
Thy_2-6			
(1) CA medium.....	<0.02	0.76	69.0
(2) CA + 0.2% DR.	<0.02	5.2	110.0
Thy_2-21			
(1) CA medium.....	7.8	0.2	126.0
(2) CA + 0.2% DR.	6.6	3.4	160.0
Thy_2-42			
(1) CA medium.....	0.02	0.05	96.0
(2) CA + 0.2% DR.	10.6	3.5	90.0

[a] Mutants were grown, in the medium indicated, as described in Materials and Methods. CA medium is Casamino Acids medium buffered at *p*H 7.0 with phosphate; DR is 2-deoxyribose.

[b] DR5P aldolase and DR kinase were assayed as described in Table 1.

[c] 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, *p*H 7.4, and 20 μmoles of Na arsenate. The reaction was run at 37 C, and samples were removed at 0, 5, 10, and 20 min. One ml of 0.3 N NaOH was added to stop the reaction. The increase in optical density was measured at 300 mμ. One unit is defined as the number of micromoles of thymine formed per hour at 37 C. An extinction coefficient of 4.04 was used.

Interrupted mating experiment. Mutant dxr-41, a transport-negative mutant, and mutant dxr-44a, a kinase-deficient mutant, were used as F^- recipients in a mating experiment with Zinder's strain Hfr A and strain Hfr B_3 of Sanderson (12). The deoxyribose genes (kinase and permease) entered the recipients in about 54 to 59 min with Hfr A and 58 to 60 min with Hfr B_3. This tentatively places the kinase gene and permease gene at about 36 to 45 min on the *Salmonella* chromosomes. A more definite map position awaits experiments involving other genetic markers. The aldolase genes in *Salmonella* have not as yet been mapped.

DISCUSSION

Cells of *S. typhimurium* grown in the presence of deoxyribose contain: (i) increased levels of DR5P aldolase, which catalyzes the reversible cleavage of deoxyribose-5-phosphate to acetaldehyde and D-glyceraldehyde-3-phosphate; (ii) increased levels of DR kinase, which catalyzes the phosphorylation of 2-deoxyribose in the presence of ATP and magnesium ion; and (iii) increased levels of thymidine phosphorylase, which catalyzes the reversible formation of thymidine from thymine and deoxyribose-1-phosphate. The relationships of these enzymes

TABLE 3. *Phenotype of low thymine-requiring mutants*

Mutant	Deoxyribose kinase	DR5P aldolase-I	Thymidine phosphorylase	DR5P aldolase-II
Thy_2-1	Inducible	Inducible	Inducible	Inducible
Thy_2-6	Inducible	Negative	"Constitutive"	Negative
Thy_2-21	Inducible	Negative	"Constitutive"	"Constitutive"
Thy_2-42	Inducible	Inducible	"Constitutive"	Negative

and their substrates is shown in Fig. 5. It seems clear that in the wild-type strain deoxyribose, deoxyribose-5-phosphate, and thymidine can all act as inducers of DR permease, DR kinase, and DR5P aldolase, type I, by their conversion to deoxyribose. It is also clear that all three substrates can act as inducers of thymidine phosphorylase, DR5P aldolase, type II, and probably phosphodeoxyribomutase by their conversion to deoxyribose-5-phosphate (the level of DRP mutase in the cells has not been determined).

In light of the data presented here, the following working model is proposed for the 2-deoxyribose gene-enzyme complex in *S. typhimurium* (Fig. 6). 2-Deoxyribose induces a specific permease, kinase, and aldolase, all of which are controlled by the same repressor. The induced DR kinase level increases the level of deoxyribose-5-phosphate in the cell, which results in the induction of a second "operon" containing information for thymidine phosphorylase and DR5P aldolase, type II. The second "operon" can be induced by adding deoxyribose-5-phosphate directly to the cells (*see* Table 4).

The following data strongly support this model, which predicts two forms of DR5P aldolase. (i) The diphasic induction curve (Fig. 4) of DR5P aldolase in the wild-type strain grown in the presence of deoxyribose suggests the presence of two forms of the enzyme. (ii) The lack of DR5P aldolase-negative mutants among the deoxyribose-negative mutants also suggests the presence of two enzymes catalyzing the same reaction. Although DR5P aldolase-negative mutants would not have been isolated on the Endo-deoxyribose plates where they are strongly inhi-

TABLE 4. *Induction by deoxyribose-5-phosphate and deoxyribose*

Strain[a]	DR5P aldolase[b]		Deoxyribose kinase[b]		Thymidine phosphorylase[c]
	units/mg		*units/mg*		*units/mg*
Wild type (C^+dg^{r-1})					
(1) CA medium	1.0		0.3		2.7
(2) CA + 0.1% DR	28.0		5.4		56.0
(3) CA + 0.1% DR5P	10.5		1.1		40.0
Mutant dxr-1	(1)	(2)	(1)	(2)	(1)
(1) CA medium	1.0	—	0.2	—	—
(2) CA + 0.1% DR	1.0	3.0	0.2	—	4.6
(3) CA + 0.1% DR5P	12.0	15.0	0.06	0.23	38.5

[a] Strains were grown and induced as indicated. CA medium is Casamino Acids medium buffered at *p*H 7.0 with phosphate.
[b] DR kinase and DR5P aldolase were assayed as described in Table 1.
[c] Thymidine phosphorylase was assayed as described in Table 2.

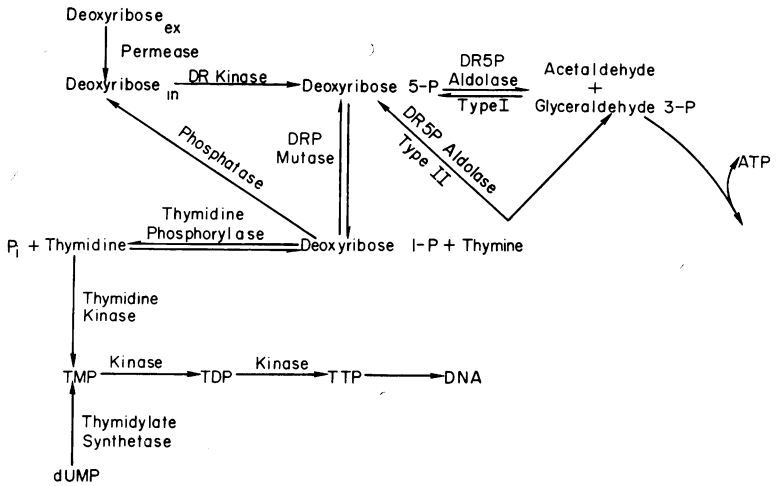

FIG. 5. *Inter-related pathways for the metabolism of 2-deoxyribose in Salmonella typhimurium.*

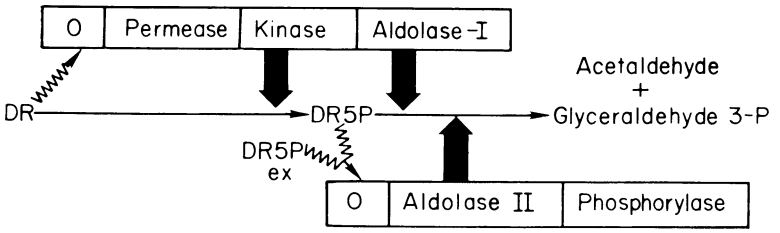

FIG. 6. *Proposed model for the Salmonella typhimurium-2-deoxyribose gene-enzyme complex. The site of action of the inducers is represented by O; permease represents the gene for deoxyribose permease; kinase represents the gene for deoxyribose kinase; aldolase I and aldolase II represent the genes for DR5P aldolase, type I and type II; and phosphorylase represents the gene for thymidine phosphorylase.*

bited, they should have been found by the technique of replica plating. If there are two forms of DR5P aldolase, selection of a DR5P aldolase-negative strain would require a double mutation, the frequency of which would be extremely low even with ethylmethane sulfonate as a mutagen. (iii) The low levels of DR5P aldolase found in some of the DR kinase-negative mutants can now be interpreted as due to the lack of induction of DR5P aldolase, type II. In the absence of DR kinase, the level of deoxyribose-5-phosphate in the cell would be lower, and the second "operon" would not be induced in the presence of deoxyribose. Those deoxyribose kinase-deficient mutants with nearly normal levels of DR5P aldolase had low but measurable levels of deoxyribose kinase. These low levels are probably sufficient for the induction of DR5P aldolase, type II. (iv) The most convincing evidence to date to support the model are the characteristics of the various types of low thymine-requiring mutants (Tables 2 and 3). Thy_2-1 is similar to the wild-type strain in the sense that DR kinase, DR5P aldolase, type I, thymidine phosphorylase, and DR5P aldolase, type II, are all induced in the presence of deoxyribose. Thy_2-6 was negative for both aldolases and appears to be a double mutant induced by nitrosoguanidine. (Thy_2-6 was isolated as a low thymine requirer directly from the aminopterin experiment.) DR kinase in this mutant is inducible, whereas the thymidine phosphorylase appears "constitutive." (It is "constitutive" in a phenotypic sense rather than in a genetic sense. The operon is probably self-induced by high levels of deoxyribose-5-phosphate in the cells). Thy_2-21 appears to be lacking DR5P aldolase, type I, since it is inducible for DR kinase but "constitutive" for both thymidine phosphorylase and DR5P aldolase, type II. In this mutant, the presence of deoxyribose in the growth medium did not affect the level of DR5P aldolase, which was about 25% of that found in the induced wild-type strain. Thy_2-42 appears, on the other hand, to be lacking DR5P aldolase, type II. This mutant has an inducible DR kinase and DR5P aldolase, type I, but is "constitutive" for the thymidine phosphorylase.

It would appear, therefore, that there are two forms of DR5P aldolase, one under the same regulation as DR kinase, namely type I, and the second, type II, under the same regulation as thymidine phosphorylase. The final proof that two types of DR5P aldolase exist in *S. typhimurium* awaits the purification and characterization of both types.

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