L-Ribulokinase and the formation of D-xylulose phosphate in *Lactobacillus pentosus*

Isomerization of L-arabinose to L-ribulose (L-*erythro*-pentulose), the first step in the fermentation of L-arabinose by *Lactobacillus pentosus*, was demonstrated by LAMPEN[1]. SIMPSON AND WOOD[2] have reported the purification of L-ribulokinase from *Aerobacter aerogenes* and have also provided evidence for the conversion of L-ribulose phosphate to D-xylulose phosphate (D-*threo*-pentulose). We have now purified L-ribulokinase from extracts of *L. pentosus* and have isolated and identified the reaction product, L-ribulose phosphate. We have also shown that extracts of *L. pentosus* obtained from cells grown on L-arabinose catalyze the reversible conversion of L-ribulose phosphate to D-xylulose phosphate.

Preparations of L-ribulokinase, purified 100-fold from the extracts of *L. pentosus*, show negligible activity with L-arabinose, D-xylose, D-ribose and D- or L-xylulose; however, they are active with D-ribulose (Table I). The ratio of activities with D- and L-ribulose was 4:1 in the crude extract and remained constant throughout the purification procedure.

TABLE I

SPECIFICITY OF PURIFIED L-RIBULOKINASE FROM *L. pentosus*

Each reaction was carried out for 15 min at 35° in 0.75 ml with 5 μmoles pentose, 12.5 μmoles ATP, 40 μmoles tris(hydroxymethyl)aminomethane buffer (pH 7.0), 5 μmoles $MgCl_2$, 12.5 μmoles NaF, 15 μmoles glutathione and 100 μg of an ammonium sulfate fraction from *L. pentosus*. A control was run without pentose. The amount of ADP formed was measured by PEP-kinase and lactic dehydrogenase[3].

Substrate	*μmoles/15 min*
L-Ribulose	2.00
D-Ribulose	0.50
L-Arabinose	0.00
D-Xylulose	0.09
L-Xylulose	0.09

For identification of the phosphate ester, the reaction was carried out at 35° in 30 ml with 200 μmoles L-ribulose, 500 μmoles ATP*, 1600 μmoles tris(hydroxymethyl)aminomethane buffer (pH 7.0), 200 μmoles $MgCl_2$, 500 μmoles NaF, 600 μmoles glutathione and 1.4 mg enzyme. After 20 min, 200 μmoles ADP were formed. Adenine nucleotides were removed from the incubation mixture by adsorption on acid-washed charcoal, and the barium salt of the phosphate ester was precipitated at pH 6.5 with 80% ethanol. 75 μmoles of the dried barium salt were dissolved in cold 0.02 *M* acetic acid, converted to the sodium salt, and hydrolyzed with potato acid phosphatase[4]. The sugar behaved in paper chromatography and in the cysteine-carbazole[5] test as did authentic ribulose; no trace of xylulose or other ketose was detected. Identity with L-ribulose was proved quantitatively by assay with L-arabinose isomerase, which is specific for L-ribulose. On incubation with this enzyme, 90% of the cysteine-carbazole reactive substance disappeared owing to its conversion to L-arabinose, exactly as with an authentic sample of L-ribulose.

In extracts of *L. pentosus* grown on L-arabinose, L-ribulose phosphate is converted to an equilibrium mixture containing nearly equal amounts of L-ribulose phosphate and D-xylulose phosphate. Essentially the same equilibrium mixture is formed with authentic D-xylulose phosphate as the substrate (Fig. 1). The formation of D-xylulose-5-phosphate from L-ribulose phosphate was established by (1) its conversion to acetyl phosphate and triose phosphate with phosphoketolase[6] and (2) paper chromatography and reactivity of the sugar with purified D-xylulokinase[7] following enzymic hydrolysis of the phosphate group. The product formed from D-xylulose phosphate in this reaction was identified as L-ribulose phosphate, using the methods described above for the kinase reaction product.

The enzyme which catalyzed this conversion can be assayed with L-ribulose phosphate as the substrate using phosphoketolase[6] to measure the formation of D-xylulose phosphate.

* The following abbreviations have been used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; PEP, phosphoenolpyruvate, ThPP, thiamin pyrophosphate.

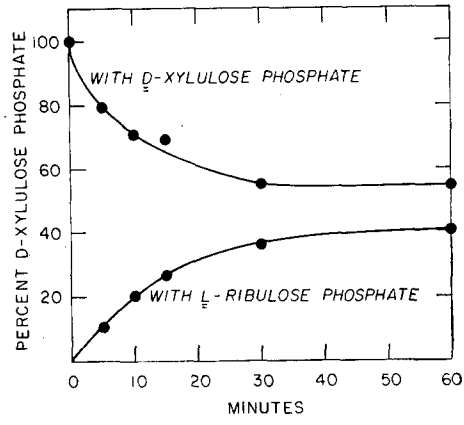
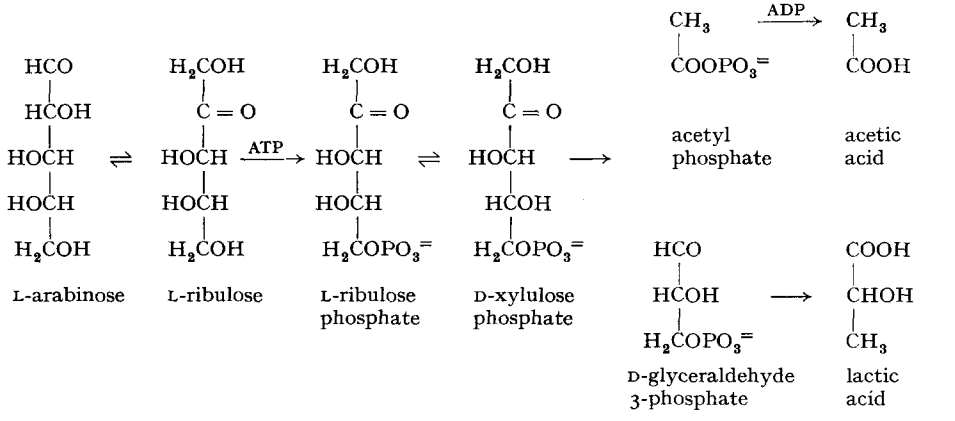


Fig. 1. Interconversion of L-ribulose phosphate and D-xylulose phosphate by an *L. pentosus* enzyme. The reaction was carried out at 35° in 3.4 ml with 9 μmoles L-ribulose phosphate or D-xylulose phosphate[8], 200 μmoles tris(hydroxymethyl)aminomethane buffer (pH 7.0) and 80 μg of an ammonium sulfate fraction. 0.4 ml aliquots were analyzed for xylulose phosphate with phosphoketolase as follows. Each was treated with 0.02 ml 0.1 *M* phosphate (pH 6.0) and 0.05 ml 0.5 *M* succinate buffer (pH 6.0) and heated at 100° for 1 min. Then 3 μmoles $MgCl_2$, 9 μmoles glutathione, 0.1 μmoles ThPP and 0.37 mg phosphoketolase were added to each, and the final volume made up to 1 ml. After incubation for 20 min, when the reaction was complete, acetyl phosphate formed was measured as described by LIPMANN AND TUTTLE[9]. At 60 min the remaining incubation mixtures were treated with potato acid phosphatase and the mixtures were assayed for L-ribulose with L-arabinose isomerase and for D-xylulose with D-xylulokinase[7]. The values were in good agreement with those calculated from the phosphoketolase assay.

Since *Lactobacillus pentosus* grown on L-arabinose is known to have the enzyme phosphoketolase, which splits D-xylulose phosphate to acetyl phosphate and triose phosphate, the sequence of reactions in the fermentation of L-arabinose by this organism can now be written as follows:



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