0021-9193/78/0133-0413$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 413-414

Vol. 133, No. 1
Printed in U.S.A.

Pentose Metabolism in *Mycobacterium smegmatis*: Comparison of L-Arabinose Isomerases Induced by L-Arabinose and D-Galactose

KEN IZUMORI,* YOUKO UEDA, AND KEI YAMANAKA

Department of Food Science, Kagawa University, Miki-cho, Kagawa-ken, 761-07, Japan

Received for publication 11 August 1977

D-Galactose, which did not serve as a growth substrate, was found to induce an L-arabinose isomerase of similar properties to the L-arabinose-induced L-arabinose isomerase. In both cases the pH profiles, pH stability, optimum temperature, heat stability, substrate specificity, metal ion requirements, mobility on polyacrylamide gel electrophoresis, and kinetic properties of the induced isomerases were identical. It appears possible that D-galactose was incorporated into the cells by an L-arabinose permease system that was also induced by D-galactose.

In a previous paper we reported that *Mycobacterium smegmatis* seemed to be unusual among microorganisms in that the inducer specificity of pentose isomerases was relatively broad (4). We discussed the configuration necessary for the induction of D-ribose, L-arabinose, and D-xylose isomerases (3, 4). D-Ribose isomerase was purified and crystallized from cells of *M. smegmatis* grown on either D-ribose or L-rhamnose. By comparing various properties of the purified enzymes, we concluded that both enzymes were present in the same protein (2). On the other hand, L-arabinose isomerase was induced by L-arabinose, D-galactose, D-fucose, dulcitol, and L-arabitol. Of various inducers, D-galactose, D-fucose, and dulcitol did not serve as growth substrates and were not substrates for the L-arabinose isomerase that they induced. It was of interest to determine whether the isomerase activities induced by these compounds were the result of the same enzyme as was induced by L-arabinose. In this paper we present a comparison of the properties of the D-galactose-induced and L-arabinose-induced L-arabinose isomerases.

M. smegmatis was grown in peptone-yeast extract medium (4) containing 0.5% L-arabinose or 0.2% D-galactose with 0.5% glycerol as growth substrate. The procedure for purification of the enzymes was almost the same as for the purification of D-ribose isomerase (5). Both enzymes were extracted from the cells by sonic treatment and purified by precipitation with polyethylene glycol, followed by chromatography on diethylaminoethyl-Sephadex A-50 and gel filtration on Sephadex G-200. By the use of these procedures, the isomerases were purified about 50-fold. The specific activities of the purified enzymes from L-arabinose- and D-galactose-grown cells were 50.2 and 31.0 U/mg of protein, respectively.

Various properties of the purified isomerases were examined and are shown in Table 1. Optimum pH, pH stability, optimum temperature, heat stability, substrate specificity, metal ion requirements, and kinetic properties were iden-

TABLE 1. *Properties of L-arabinose isomerases induced by L-arabinose and D-galactose*[a]

Properties	L-Arabinose isomerase	
	L-Arabinose induced	D-Galactose induced
Optimum pH	7.0–7.5	7.0–7.5
pH stability[b]	7.5–10.5	7.5–10.5
Optimum temp[c] (°C)	45	45
Heat stability[d]	45°C, 10 min	45°C, 10 min
Substrate specificity[c]	L-Arabinose	L-Arabinose
K_m values for L-arabinose[c] (mM)	33.3	27.0
Metal ion requirement[e]	Mn^{2+}, Co^{2+}, Mg^{2+}	Mn^{2+}, Co^{2+}, Mg^{2+}
K_i values for[c] (mM)		
L-Arabitol	2.17	2.07
Ribitol	1.25	1.15

[a] The enzyme assay was carried out as described in a previous paper (4).

[b] The enzyme was kept at 2°C for 3 days, and remaining activity was determined. In alkaline pH, both enzymes were stable.

[c] The reaction was carried out in 0.025 M Tris-hydrochloride [tris(hydroxymethyl)aminomethane-hydrochloride] buffer (pH 7.5) in the presence of 0.5 mM $MnCl_2$.

[d] Both enzyme activities were stable against heating for 10 min at 45°C (pH 7.5) but decreased by 50 and 100% upon heating for 10 min at 50 and 55°C, respectively.

[e] The enzymes did not lose activity during dialysis against 0.05 M Tris-hydrochloride buffer containing 1 mM EDTA (ethylenediaminetetraacetic acid) for 2 days. The enzyme activity was inhibited by the addition of 10^{-4} M EDTA into the reaction mixture, and addition of 10^{-4} M Mn^{2+}, Co^{2+}, or Mg^{2+} restored the activity.

tical in both enzymes. D-Galactose was an inducer of the isomerase but was not a substrate for either of the enzymes. Both purified preparations contained only one significant contaminating protein, as shown in photographs of the bands from polyacrylamide gel electrophoresis (Fig. 1). The contaminating protein had almost the same mobility as L-arabinose isomerase on a diethylaminoethyl-Sephadex A-50 column and after gel filtration on Sephadex G-200. After extraction of gel slices with buffer, L-arabinose isomerase activity was assayed, and the activity was only found in the major band, which is indicated by an arrow in Fig. 1. Apparently, both isomerase activities result from enzymes possessing the same electrophoretic mobility. The molecular weights of these enzymes were almost identical, as indicated by the gel filtration profile, using a Sephadex G-200 column.

Although the induction specificity of L-arabinose isomerase in *M. smegmatis* was unusual among microorganisms, the properties of the L-arabinose isomerase induced were almost the same as those reported in various other microorganisms (6).

As mentioned above, *M. smegmatis* was unable to utilize D-galactose as a sole source of carbon and energy for growth, but D-galactose must have been incorporated into the cells to induce L-arabinose isomerase. D-Galactose permease activity in cells that had been grown on various substrates was determined by using D-[^{14}C]galactose. Uptake activity of D-galactose in cells grown on L-arabinose or D-galactose was about six times higher than that in cells grown only on glycerol (Table 2). The uptake activity in cells grown on D-galactose was inhibited strongly by the addition of nonradioactive L-arabinose or D-galactose. From these results, it appears possible that the same permease system was induced by either D-galactose or L-arabinose.

On the basis of the above results, D-galactose was found to induce both an L-arabinose permease and an L-arabinose isomerase. The isomerase induced by D-galactose had similar properties to the L-arabinose-induced L-arabinose isomerase. D-Galactose was able to be a substrate of the permease, but not a substrate of the isomerase.

TABLE 2. *D-Galactose uptake activity in cells of M. smegmatis grown on various substrates*[a]

Growth medium	D-Galactose uptake activity (μmol/g [dry wt] per min)		
	No addition	Addition of unlabeled:	
		D-Galactose (20 mM)	L-Arabinose (20 mM)
Glycerol (0.5%)	0.25		
L-Arabinose (0.5%)	1.40		
Glycerol (0.5%) + D-galactose (0.2%)	1.87	0.05	0.22

[a] The commercial D-[^{14}C]galactose was used without further purification. The assay mixture contained 2 mM D-[^{14}C]galactose and about 10 mg (dry weight) of washed cells in 2 ml of mineral salt medium. After incubation at 35°C for 10 min, the assay mixture was filtered through a paper disk (Toyo no. 2). The paper was washed with 10 ml of the mineral salt medium not containing ^{14}C-labeled compounds. The radioactivity retained on the paper was measured in a gas-flow GM counter. In inhibition studies, 20 mM unlabeled D-galactose or L-arabinose was added simultaneously in the assay mixture with D-[^{14}C]galactose.

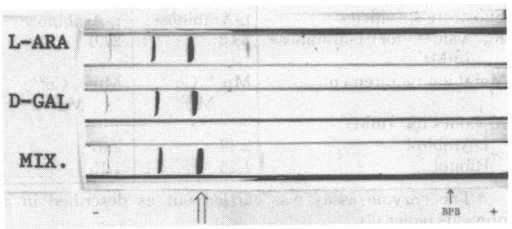

FIG. 1. *Polyacrylamide gel electrophoresis of purified L-arabinose isomerases. Electrophoresis was done as described by Davis (1). L-ARA: A 10-μg portion of purified L-arabinose-induced L-arabinose isomerase; D-GAL: 10 μg of purified D-galactose-induced L-arabinose isomerase; MIX.: 10 μg of each of the two L-arabinose isomerase preparations were layered onto the gels. The band of L-arabinose isomerase is indicated by the arrow.*

This work was supported by grant 266049 from the Japanese Ministry of Education.

LITERATURE CITED

1. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404–427.
2. **Izumori, K., M. Mitchell, and A. D. Elbein.** 1976. Evidence that the isomerization of D-ribose and L-rhamnose is catalyzed by the same enzyme in *Mycobacterium smegmatis*. J. Bacteriol. **126**:553–555.
3. **Izumori, K., and K. Yamanaka.** 1977. Speculative studies on an anomeric specificity of inducers of D-xylose isomerase. FEBS Lett. **77**:133–135.
4. **Izumori, K., K. Yamanaka, and A. D. Elbein.** 1976. Pentose metabolism in *Mycobacterium smegmatis*: specificity of induction of pentose isomerases. J. Bacteriol. **128**:587–591.
5. **Izumori, K., A. W. Rees, and A. D. Elbein.** 1975. Purification, crystallization and properties of D-ribose isomerase from *Mycobacterium smegmatis*. J. Biol. Chem. **250**:8085–8087.
6. **Noltman, E. A.** 1970. Aldose-ketose isomerases, p. 271–354. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 1. Academic Press Inc., New York.