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# Evidence that the Isomerization of D-Ribose and L-Rhamnose Is Catalyzed by the Same Enzyme in *Mycobacterium smegmatis*

KEN IZUMORI, MIKE MITCHELL, AND ALAN D. ELBEIN*

*Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284*

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D-Ribose isomerase was purified and crystallized from cells of *Mycobacterium smegmatis* grown on either D-ribose or L-rhamnose. Isomerase activity for both of these sugars remained together throughout the purification. The isomerase from L-rhamnose-grown cells had the same chemical and physical properties as the enzyme isolated from D-ribose grown cells. In addition, immunological studies indicated that both activities were in the same protein since antisera prepared against either of the crystals cross-reacted with the other and gave lines of symmetry by the agar gel diffusion method.

D-Ribose isomerase was previously purified and crystallized from cells of *Mycobacterium smegmatis* grown on D-ribose (2). This enzyme was active on L-rhamnose as well as on D-ribose. Since isomerase activity for these two sugars was also observed in cells grown on L-rhamnose, it was of interest to determine whether the isomerase activity induced by L-rhamnose was due to the same enzyme as that induced by D-ribose.

*Mycobacterium smegmatis* was grown in nutrient broth containing either 0.5% L-rhamnose, 0.5% D-ribose, or some other sugar. In either case, isomerase activity was purified and crystallized as previously described (2). Isomerase activity, induced by either substrate, was purified and crystallized using the identical procedure. In either case, the crystals were microscopically indistinguishable. In addition, both activities copurified and the ratio of the two activities remained constant throughout purification. The properties of the isomerase induced by L-rhamnose were compared to those of the D-ribose-induced enzyme. In both cases, the pH profiles, substrate specificity, metal ion requirements, and kinetic properties were identical.

Conclusive proof that both activities reside in the same protein molecule was obtained by immunological studies. Antisera were prepared against ribose-induced and rhamnose-induced isomerase by injecting rabbits with 1 mg of crystalline enzyme followed in 2 weeks by a second injection of 1 mg of crystalline enzyme. Six days later, rabbits were bled and sera were collected. As shown in Fig. 1, antisera prepared against the D-ribose-induced isomerase reacted with the crystalline enzyme preparation induced by either D-ribose or L-rhamnose. Furthermore, the precipitin lines were continuous indicating the presence of a single protein. Likewise, Fig. 2 shows that the reverse was also true; antisera prepared against the L-rhamnose-induced isomerase reacted with both enzyme preparations and gave a continuous precipitation line.

Cells were grown in Trypticase soy broth and

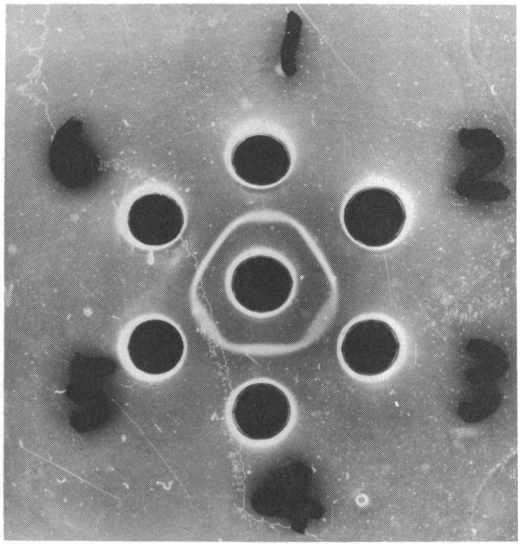

FIG. 1. *Immunodiffusion of D-ribose isomerases. Experimental conditions were described in Materials and Methods. Center well, Rabbit antiserum against D-ribose-induced D-ribose isomerase; 1,3,5, D-ribose-induced enzyme; 2,4,6, L-rhamnose-induced enzyme.*

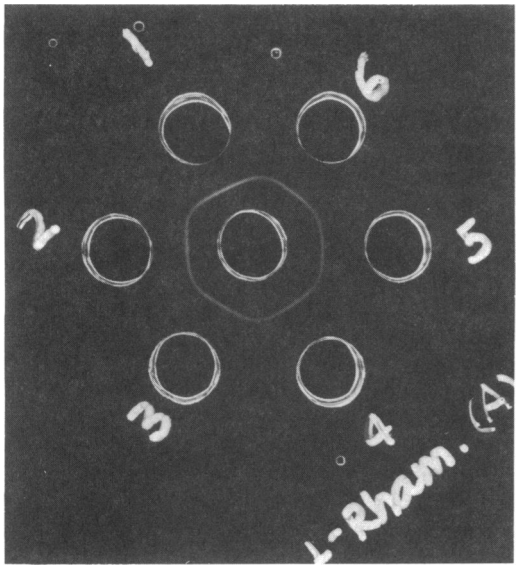


FIG. 2. *Immunodiffusion of* D-*ribose isomerases. Center well, rabbit antiserum against* L-*rhamnose-induced* D-*ribose isomerase; 1,3,5,* D-*ribose-induced enzyme; 2,4,6,* L-*rhamnose-induced enzyme.*

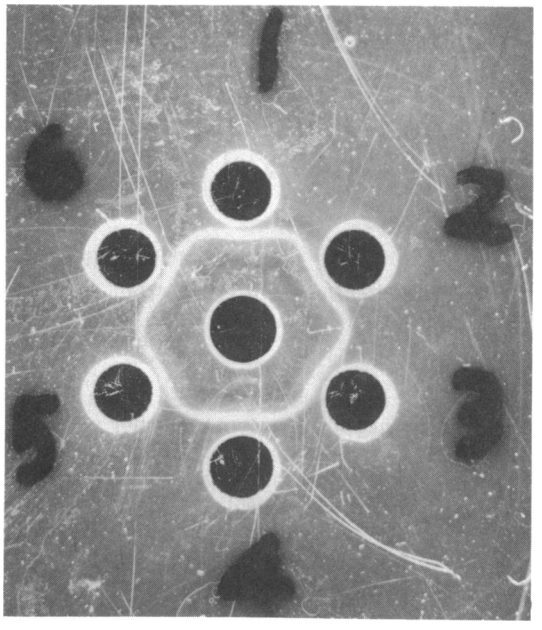


FIG. 4. *Immunodiffusion of crude enzymes. Center well, Rabbit antiserum against* L-*rhamnose-induced* D-*ribose isomerase. 1,4,* D-*ribose-; 2,5,* L-*lyxose-; 3,6,* L-*rhamnose-induced crude enzymes.*

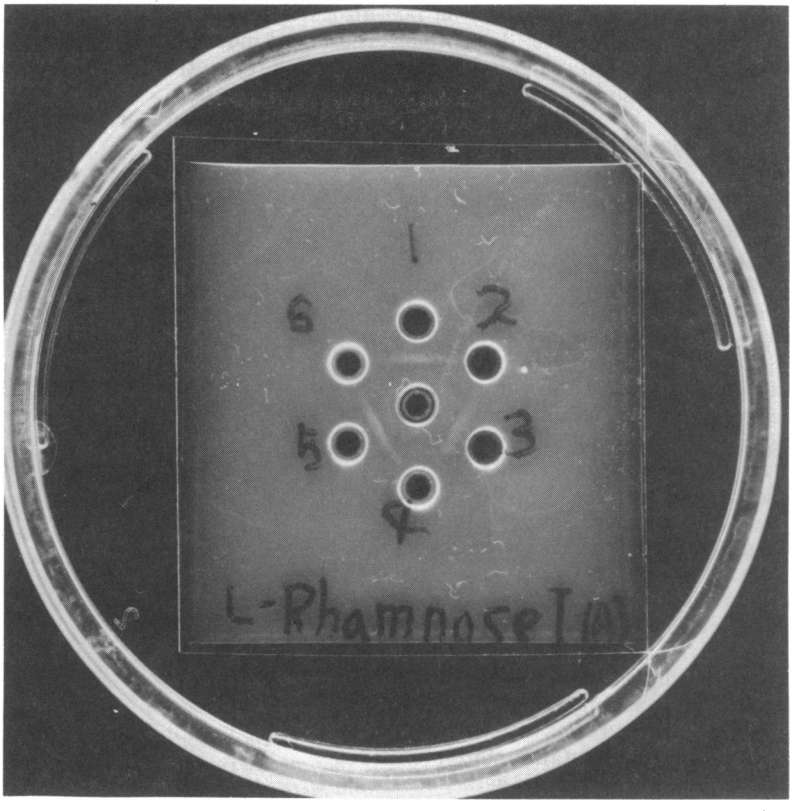


FIG. 3. *Immunodiffusion of crude extracts of cells incubated with various sugars. Center well, Rabbit antiserum against* L-*rhamnose-induced* D-*ribose isomerase. 1,* D-*Ribose-; 2,* D-*arabinose-; 3,* L-*lyxose-; 4,* L-*arabinose-; 5* L-*rhamnose-; and 6* D-*xylose-induced crude extracts.*

then placed in nutrient broth in the presence of D-ribose, L-rhamnose, and L-lyxose, three sugars that induce the D-ribose isomerase, as well as D-arabinose, L-arabinose, and D-xylose, three sugars that do not induce this enzyme. The crude extracts prepared from each of these cultures were then tested against antiserum prepared against the D-ribose-induced isomerase. As shown in Fig. 3, extracts of D-ribose-, L-rhamnose-, and L-lyxose-grown cells reacted with the antisera, whereas extracts from cells grown in the other sugars did not. Furthermore, as indicated in Fig. 4, the extracts from ribose-, rhamnose-, and lyxose-grown cells gave a single precipitin line of symmetry indicating that they each contained the same protein. These data indicate that the same protein molecule catalyzes the isomerization of D-ribose, L-rhamnose, and L-lyxose.

L-Rhamnose isomerase was previously isolated from *Escherichia coli* (3) and *Lactabacillus plantarum* (1), but these enzymes were not tested for activity towards D-ribose. We found that cells of *E. coli* K-12 or *Aerobacter aerogenes* PPL-R3 grown on L-rhamnose had isomerase activity for both L-rhamnose and D-ribose. However, when these two organisms were grown in the presence of D-ribose, no isomerase activity could be detected towards either substrate.

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