Short Communication

Induction of D-Ribose Isomerase by L-Ribose in *Mycobacterium smegmatis*Ken IZUMORI,*¹ Shigeru SUGIMOTO*²
and Burckhard KRASKA*Department of Food Science, Kagawa University,
Miki-cho, Kagawa-ken 761-07, Japan*Institut für Organische Chemie und Biochemie,
Technische Hochschule Darmstadt,
D-6100 Darmstadt, FRG

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We have previously reported that *Mycobacterium smegmatis* seems to be unusual among microorganisms in that the inducer specificity of pentose isomerases is relatively broad.¹⁾ D-Ribose isomerase is induced by D-ribose, L-lyxose and L-rhamnose, the enzymatic activity residing in the same protein.²⁾ In continuing our investigations, we have checked the activity of the hitherto unavailable L-ribose which itself was not a substrate of the enzyme and also not a growth substrate for the organism. The sugar, like the enantiomeric D-ribose induced the same D-ribose isomerase possibly due to the characteristic configuration and conformation of the ribose molecule.

The results are shown in Fig. 1. L-Ribose, prepared by the method of Austin and Humoller³⁾ was used in induction experiments. D-Ribose and L-ribose induced D-ribose isomerase of 0.0058 and 0.0060 unit per mg of protein in 8 hours, respectively. However, D-lyxose, L-arabinose, and D-xylose had no effect on D-ribose isomerase induction. D-Ribose isomerase induced by L-ribose reacted against antiserum prepared against the D-ribose induced isomerase giving a precipitin line of symmetry. This provides evidence that the biosynthesis of the same protein is

FIG. 1. Immunodiffusion of Crude Extracts of Cells Incubated with Various Pentoses.

Central well rabbit antiserum against D-ribose induced crystalline D-ribose isomerase. 1, D-Ribose; 2, L-ribose; 3, D-lyxose; 4, L-arabinose; 5, D-xylose; and 6, L-ribose induced crude extracts. L-Ribose was prepared according to Austin and Humoller.³⁾ Other experimental conditions were the same as previously reported.²⁾

induced likewise by a deoxyhexose (L-rhamnose), D- and L-ribose and L-lyxose, none of the other pentoses acting as inducer of this special isomerase.²⁾ By inspection of molecular models of various inducers, we have been able to reveal the common structure required for the induction of *Mycobacterium smegmatis* pentose isomerases.⁴⁾ In the case of the D-lyxose isomerase, the configuration of the hydroxy groups at C-1, C-2 and C-3 of β -D-lyxose or its structural analogy seems to be indispensable for an induction of the enzyme. Considering the pentopyranose conformers in aqueous solution and their stereochemical interrelation, we discussed the unexpected activity of L-ribose in the D-ribose isomerase induction.

The preferred conformations predicted by theory⁵⁾ and in most cases experimentally substantiated by ¹H- and ¹³C-NMR-spectroscopy^{6,7)} are given in Fig. 2. It is noteworthy that several pentoses relevant to the enzyme-induction process discussed here, adopt exclusively either the ¹C₄ or ⁴C₁ conformation, e.g., β -D-lyxose,⁸⁾ α -D-ribose, β -D-ribose and

*¹ To whom correspondence should be addressed.

*² Present address: Taiyo Foods Co. LTD, Tsuchida 136, Okayama City, Okayama-ken 703.

PENTOSE	4C_1 CONFORMERS				1C_4 CONFORMERS			
	α Configuration Group A Group B		β Configuration Group A Group B		α Configuration Group A Group B		β Configuration Group A Group B	
D-RIBOSE								
L-RIBOSE								
D-ARABINOSE								
L-ARABINOSE								
D-XYLOSE								
L-XYLOSE								
D-LYXOSE								
L-LYXOSE								

FIG. 2. Conformational Presentation of the Eight Pentopyranoses.

Group B depicts forms which arise by rotating the pyranose ring in Group A in a reference-plane (C-2, C-3, C-5 and oxygen atom) by 180° as demonstrated for β -L-ribose in Fig. 3.

β -L-ribose.^{7,9)} From the induction experiments, a contribution of furanose tautomers—though present in solution—to the enzyme induction is ruled out since structural analogies between intrinsically different molecules are highly improbable.

As already pointed out, the spatial arrangement of the C-1, C-2 and C-3 hydroxyl groups of the appropriate sugars *inter alia* is decisive for the inducer specificity of D-ribose isomerase.¹⁾ It can easily be shown (Fig. 3) that α -D-ribose, β -L-lyxose and β -L-rhamnose have an identical alignment of three adjacent centres of chirality, all sugars adopting the 1C_4 conformation exclusively. At the first glance, L-ribose does not meet this requirement. However, upon rotation of the pyranose ring in the C-2, C-3, C-5 and oxygen-atom plane,⁴⁾ the hydroxygroups appended to C-4, C-3 and C-2 of β -L-ribose overlap with the C-1, C-2 and C-3 arrangement of both α -D-ribose and β -L-lyxose (Fig. 3). None of the other pentoses displays the same structural

FIG. 3. Corresponding Structural Portions of Inducers of D-Ribose Isomerase.

A, β -L-lyxose ($R=H$), β -L-rhamnose ($R=CH_3$); B, α -D-ribose; C, β -L-ribose; D, β -L-ribose rotated.

features directly or upon rotation on the reference plane. Interestingly enough, these pentoses acting as inducers adopt the 1C_4 conformation in solution.

L-Ribose could not induce D-lyxose isomerase. In the case of D-lyxose isomerase the steric alignment at C-1, C-2 and C-3 of β -D-lyxose (4C_1 conformation in solution) become superimposable on the corresponding C-4, C-3 and C-2 sequence of β -D-ribose (Group B, 4C_1) and the C-1, C-2 and C-3 sequence of α -L-

ribose (4C_1), thereby underlining the close resemblance in the stereochemistry of these sugars. The experimentally proven ineffectivity of L-ribose in the induction of D-lyxose isomerase shows the more severe requirement of the spatial arrangement of four hydroxyl groups for the D-lyxose isomerase induction. Namely, all of the hydroxyl groups of β -D-ribose (Group B) are able to overlap with those of β -D-lyxose completely and therefore D-ribose can induce the enzyme. However, one of the four hydroxyl groups (C-4) of α -L-ribose is not superimposable on that of β -D-lyxose and so L-ribose is ineffective in the induction of D-lyxose isomerase.

Thus, D-ribose isomerase is induced by α -D- and β -L-ribose (Group B) adopting the 1C_4 conformation, while the D-lyxose isomerase is induced by β -D-lyxose and β -D-ribose (Group B) in the 4C_1 conformation.

Furthermore, it is reasonable to assume that the spatial arrangement of certain sugar alcohols in their zig-zag¹⁰⁾ or sickle¹¹⁾ conformation fortuitously meets the required structure as the enzyme inducer. Thus the inducer activity of dulcitol in D-lyxose isomerase¹⁾ may be explained.

We hope that these observations may contribute to a some what better understanding of the mechanism of enzyme induction in microorganisms.

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