D-Ribose Competitively Reverses Inhibition by D-Psicose of Larval Growth in *Caenorhabditis elegans*

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D-Psicose inhibits the growth of L1 stage *Caenorhabditis elegans*. Sugars, involved in the pentose phosphate pathway, were examined for their ability to reverse the inhibition. Among these sugars, D-ribose specifically exerted reversing activity in a competitive manner. The ingested sugars are probably phosphorylated, although it remains to be seen whether D-psicose is phosphorylated. The structural similarity of D-psicofuranose 6-phosphate (Pf6P) or D-psicofuranose (Pf) to D-ribofuranose 5-phosphate (Rf5P) suggests that Pf6P or Pf is reversibly docked in the active site(s) of ribose-5-phosphate isomerase(s) to act as an antimetabolite to Rf5P, leading to inhibition of the biosynthesis of nucleic acids. D-Psicose was much less potent against the L4 stage than against the L1 stage. This is probably because in the L4 stage the somatic cell lineages come to an end and the number of germ-line nuclei increases to about 1000.

Key words D-ribose; D-psicose; antimetabolite; *Caenorhabditis elegans*; growth

Certain ketohexose stereoisomers have been shown to exert biological activity. L-Sorbose induces glycolytic limitation in canine erythrocytes, leading to hemolysis.¹⁾ Phosphorylation of D-fructose²⁾ or D-tagatose³⁾ in the liver causes severe metabolic disturbances, due to the accumulation of phosphorylated intermediates. It was recently shown that, among D- and L-forms of ketohexose stereoisomers (psicose, sorbose, fructose, and tagatose), as well as D-glucose and D-galactose, the rare sugar D-psicose specifically inhibited the motility, growth, and reproductive maturity of L1 stage *Caenorhabditis elegans*.⁴⁾

D-Psicose was recorded as a constituent moiety of the antibiotic psicofuranine,⁵⁾ having marked antibacterial⁶⁾ and antitumor⁷⁾ activities *in vivo*, from culture filtrates of *Streptomyces hygroscopicus* var. *decoyicus*.⁸⁾ The furanose form of D-psicose resembles in structure that of D-ribose (Fig. 1). This structural resemblance suggests an antimetabolite mode of action. The purpose of this paper is to identify the biochemical target of D-psicose in larvae of *C. elegans* by testing compounds for their ability to reverse the inhibitory activity of D-psicose and to propose a possible inhibition mechanism of D-psicose.

MATERIALS AND METHODS

Chemicals D-Ribose and sodium D-gluconate were purchased from Wako, Osaka, Japan; D-ribulose, from Omicron, South Bend, IN, U.S.A.; and D-erythrose and D-sedoheptulose, Sigma-Aldrich, St. Louis, MO, U.S.A. A variety of chemicals needed to prepare *C. elegans* cultivation and handling media⁹⁾ were obtained from Wako. All chemicals used here were of analytical grade.

L1 and L4 Larvae of *C. elegans* Standard conditions were used for *C. elegans* cultivation.⁹⁾ A stock culture of the Bristol strain of *C. elegans* was incubated at 20 °C in a liquid growth medium (LGM), *i.e.*, complete S medium supplemented with *Escherichia coli* strain OP50 (28.6 g wet weight per liter), by shaking for 7 d. Dauer stage larvae, induced by starving the culture for 14 d, were transferred onto 3.5-cm NGM agar plates with *E. coli* at 20 °C and L4 larvae were prepared after 24 h.¹⁰⁾ Eggs were collected by treating egg-bearing adults with 5.4% alkaline hypochlorite, followed by shaking the eggs in M9 buffer at 20 °C for 22 h to prepare L1 larvae.

Experiments Individual three-L1 larva sets were incubated in each of 250- μ l hemiellipsoidal wells holding 200 μ l of LGM containing D-psicose (111 mM) with and without one of the compounds to be tested for reversing activity at 20 °C for 84 h, in accordance with the previous method.⁴⁾ L1 larvae were incubated as controls in LGM. Similarly, three-L4 or -L1 larva sets were incubated in LGM (200 μ l) with and without D-psicose (167 mM) at 20 °C. Eighteen larvae were used at each concentration.

Motility, Growth, and Reproductive Parameters Nematode motility is expressed as a motility index, $\sum nN_n / \sum N_n^{4,11)}$, N_n is the number of worms showing the movements stated in $n=3$ (fully sinusoidal locomotion movements), $n=2$ (slow movements), $n=1$ (feeble wriggling of the upper or lower part), or $n=0$ (motionlessness, death). The worms in the incubation wells were kept at 4 °C for 0.5 h to depress their movements, and pictures were taken with a CCD camera attached to a microscope (Olympus BX51). Body lengths were measured by the previous method,⁴⁾ which is virtually

Fig. 1. Structures of D-Psicose and D-Ribose in the Cyclic Furanose Form

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Fig. 2. Larval Growth in LGM Containing Both D-Psicose at 111 mM and One of the Other Sugars at Concentrations Indicated in the Figure

○, D-ribose; ▲, D-ribulose; △, D-erythrose; ×, D-sedoheptulose; ●, sodium D-gluconate; ◆, larval growth in LGM containing only D-psi-cose (111 mM); and ◇, that in LGM.

identical to that described by Fujiwara *et al.*¹²⁾; the software was Mac SCOPE version 2.5 (Mitani, Tokyo, Japan). The egg-bearing rate is given as a percentage of worms bearing eggs in the uterus.

RESULTS

Several sugars, involved in the pentose phosphate pathway, were examined for reversal of inhibition by D-psi-cose (111 mM) of larval growth during a period of 84 h (Fig. 2). D-Ribulose, sodium D-gluconate, D-erythrose, and D-sedoheptulose were all ineffective in reversing the inhibition by D-psi-cose. In contrast, D-ribose did reverse activity (○ in Fig. 2). At 160 mM, mean body length $\pm$ S.D. of worms ($N=18$) reached $905 \pm 124 \mu\text{m}$ after 84 h; *i.e.*, the mean value was 2.7 times that (◆ in Fig. 2) of worms incubated in LGM containing only D-psi-cose (111 mM), or 82% that (◇ in Fig. 2) of controls in LGM. In addition, after 84 h the initial motility index of 3 decreased to 2.9 ± 0.2 only, and $61 \pm 25\%$ of L1 larvae became egg-bearing adults. For each D-ribose concentration, the D-psi-cose concentration against body length was plotted into a straight line, which was approximately parallel with the line given by worms in LGM containing only D-psi-cose (Fig. 3). The results show that D-psi-cose's inhibition of larval growth decreased when the concentration of D-ribose added to LGM increased. The body length for a ratio of D-ribose to D-psi-cose (1 : 1) was almost constant, 860–884 μm (Table 1).

Furthermore, D-psi-cose (167 mM) was examined for its potency against the reproductive maturity of L4 larvae in LGM. During a period of 30 h, this sugar caused a slight reduction of egg-bearing rates only, and so all L4 larvae became egg-bearing adults within 36 h, compared with 30 h for controls (Fig. 4).

Fig. 3. Larval Growth in LGM Containing Both D-Psicose at Concentrations Indicated in the Figure and D-Ribose at 0 mM (△), 60 mM (●), 80 mM (▲), and 111 mM (○)

Table 1. Larval Growth in LGM Containing D-Psicose and D-Ribose at a Ratio of 1 : 1

D-Psicose (mM)	D-Ribose (mM)	Ratio	Body length $\pm$ S.D. (μm)
111	111	1 : 1	884 ± 151
80	80	1 : 1	875 ± 136
60	60	1 : 1	860 ± 107

Fig. 4. Reproductive Maturity of L1 (●) and L4 (○) Larvae in LGM with (—) and without (---) D-Psicose at 167 mM

DISCUSSION

Among the several sugars tested here, D-ribose specifically reversed inhibition by D-psi-cose of larval growth (Fig. 2). The results, including the observations on nematode motility and egg-bearing rate, suggest that the external supply of D-ribose to larvae in LGM containing D-psi-cose considerably compensated for a deficiency in D-ribose. Therefore, D-psi-cose appears to interfere with the bioconversion of D-ribulose to D-ribose in the larvae. With competitive inhibition the reaction rate for a certain ratio of metabolite to antimetabolite is almost constant regardless of their absolute values.^{13,14)} This condition was fulfilled by D-ribose (Fig. 3, Table 1).

Ribose-5-phosphate isomerases (Rpis), which catalyze the interconversion of D-ribose 5-phosphate (R5P) and D-ribulose 5-phosphate (Ru5P),¹⁵⁾ are classified into type A (occurring in all three kingdoms of life) or type B (occurring in some

bacteria and protozoa).¹⁶⁾ The cyclic α - and β -furanose forms are much more abundant in aqueous solution than the open-chain form.¹⁷⁾ Recently, it has been proposed that the cyclic form (especially, the β form) of R5P is initially docked in the active site of RpiB from *Mycobacterium tuberculosis*, which is causative of tuberculosis, and transformed to the open-chain form, leading to the isomerization reaction to Ru5P via the high-energy intermediate 1,2-*cis*-enediolate.¹⁸⁾ A similar hypothesis has been proposed for phosphoglucose isomerase.¹⁹⁾ D-Ribose, tested here, is probably phosphorylated in the larvae, as well as other sugars. On the other hand, it remains to be seen whether D-psicose will be phosphorylated. Among the resulting phosphates, D-ribofuranose 5-phosphate (Rf5P) structurally resembles D-psicofuranose 6-phosphate (Pf6P) or D-psicofuranose (Pf). *C. elegans* possesses a gene called rpiA-1, that encodes a putative Rpi (<http://www.wormbase.org/db/gene/gene?name=WB-Gene00015101;class=Gene>). Therefore, it is possible that Pf6P or Pf is reversibly bound in the active site of the relevant larval enzyme(s), probably Rpi(s), to act as an enzyme-inhibitor. Thus, the antimetabolite mode of action appeared to be a block against the biosynthesis of nucleic acids.

D-Psicose was much less potent against the reproductive maturity of L4 larvae than against that of L1 larvae (Fig. 4). This appeared to be attributable to the fact that in the L4 stage the somatic cell lineages come to an end²⁰⁾ and the number of germ-line nuclei increases to about 1000.²¹⁾ Further investigations, including isolation of Rpi(s) from *C. elegans*, are needed to detail the inhibition mechanism of D-psicose.

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