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Growth inhibitory effect of D-arabinose against the nematode *Caenorhabditis elegans*: Discovery of a novel bioactive monosaccharide

Hirofumi Sakoguchi [a], Akihide Yoshihara [b], Tomoya Shintani [c], Kazuhiro Okuma [c], Ken Izumori [b], Masashi Sato [a,*]

[a] *Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan*
[b] *Rare Sugar Research Center, Kagawa University, Miki, Kagawa 761-0795, Japan*
[c] *Research Laboratory, Matsutani Chemical Industry Co Ltd, Itami, Hyogo 664-8508, Japan*

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ABSTRACT

Biological activities of unusual monosaccharides (rare sugars) have largely remained unstudied until recently. We compared the growth inhibitory effects of aldohexose stereoisomers against the animal model *Caenorhabditis elegans* cultured in monoxenic conditions with *Escherichia coli* as food. Among these stereoisomers, the rare sugar D-arabinose (D-Ara) showed particularly strong growth inhibition. The IC_{50} value for D-Ara was estimated to be 7.5 mM, which surpassed that of the potent glycolytic inhibitor 2-deoxy-D-glucose (19.5 mM) used as a positive control. The inhibitory effect of D-Ara was also observed in animals cultured in axenic conditions using a chemically defined medium; this excluded the possible influence of *E. coli*. To our knowledge, this is the first report of biological activity of D-Ara. The D-Ara-induced inhibition was recovered by adding either D-ribose or D-fructose, but not D-glucose. These findings suggest that the inhibition could be induced by multiple mechanisms, for example, disturbance of D-ribose and D-fructose metabolism.

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Monosaccharides exist in a variety of stereoisomer forms. However, with the exception of isomers such as D-glucose (D-Glc) and D-fructose (D-Fru), which exist in great abundance, the vast majority of their stereoisomers cannot be found naturally. It is therefore difficult to isolate them from natural sources due to their scarcity, and they are also difficult to chemically synthesize because of the complexity of their structures. As a result, the biological activities of unusual monosaccharides (rare sugars) have largely remained unstudied until recently.

Izumori developed a novel method, called the Izumo-ring strategy, for the bulk production of these rare sugars using bacterial enzymes.[1] This approach facilitates the biomedical study of these rare sugars. For example, anti-hyperglycemic[2] and anti-obesity[3] effects have been identified for D-allulose (D-Alu, also known as D-psicose), the C3 epimer of D-Fru, while in vitro anti-cancer activities[4,5] have been identified for D-allose, the C3 epimer of D-Glc. Biological activities of these rare sugars can be attributed to the properties of antimetabolites that mimic the structures of metabolizable sugars, including D-Glc and D-Fru. In order to investigate the antimetabolic activities of these rare sugars, a simple bioassay is necessary due to the sheer number of monosaccharide stereoisomers. We believe that growth assays using larvae of the nematode *Caenorhabditis elegans* are a convenient and useful primary screening method to search for biologically active rare sugars.

Caenorhabditis elegans is an attractive multicellular animal model for biological research because of its small size, simplicity, short lifespan (about 30 days), quick turnover (3 days), ease of propagation and maintenance, routine genetic manipulation, and cost-effectiveness. It has been widely used as a model system for studying development, aging, metabolism, and other physiological processes.[6–8]

In our previous report,[9] the growth inhibitory effects of all ketohexose stereoisomers, including D- and L-forms of Alu, Fru, tagatose, and sorbose, against *C. elegans* were examined in monoxenic conditions where animals were cultured in a liquid medium containing *Escherichia coli* as a food source. Of these ketohexose stereoisomers, only D-Alu inhibited nematode growth. We

Abbreviations: IC_{50}, half maximal inhibitory concentration; SD, standard deviation; ATP, adenosine triphosphate; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate.

* Corresponding author. Tel.: +81 87 891 3090; fax: +81 87 891 3021.
E-mail address: sato@ag.kagawa-u.ac.jp (M. Sato).

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performed further screening of aldopentose stereoisomers including D- and L-forms of ribose (Rib), arabinose (Ara), xylose (Xyl), and lyxose (Lyx) using the monoxenic *C. elegans* assay. Here, we report a comparison of the growth inhibitory effects of these aldopentoses against *C. elegans*, and that among them D-Ara exerted particularly strong inhibition. The inhibitory effect of D-Ara was also confirmed in axenic conditions where animals were cultured in the chemically defined medium CeMM (*C. elegans* maintenance medium)[10,11] to exclude the possible influence of *E. coli*. In addition, we discuss the mechanisms responsible for D-Ara-induced inhibition based on the results of rescue experiments by simultaneous treatment with the metabolizable sugars D-Rib, D-Glc, or D-Fru.

The structures of aldopentoses used in this study are shown in Figure 1. D-Ara,[12] D-Lyx,[13] L-Rib,[14] L-Xyl,[15] L-Lyx,[16] D-Alu,[17] and D-arabitol[13] were prepared enzymatically at the Rare Sugar Research Center, Kagawa University. Production schemes of these rare sugars are shown in Supplementary data. Purity of the sugars was confirmed to be more than 98% by HPLC and ^{13}C NMR. D-Rib, D-Xyl, L-Ara, and 2-deoxy-D-glucose (2-DG) were obtained from Wako Pure Chemical Industries (Osaka, Japan). All commercially available chemicals were of reagent grade, and were used without further purification.

The wild-type *C. elegans* strain N2 (var. Bristol) was maintained at 20 °C on nematode growth medium (NGM) seeded with *Escherichia coli* OP50 as a food source.[18] Eggs of *C. elegans* were collected by treating egg-bearing adults with alkaline hypochlorite solution, and were shaken in S basal medium at 20 °C for 24 h, to prepare first-stage larvae (L1).[18]

In our previous report,[9] we evaluated the growth of the nematodes in terms of their egg-bearing rate; for this report, however, in order to evaluate growth more precisely, we used the projected area of the nematodes as the index. The monoxenic assay was performed as follows: about twenty L1 larvae were transferred into each well of a 24-well culture plate (4846-24FS; Watson Co., Ltd, Kobe, Japan) that held 200 µl of S liquid medium[18] containing *E. coli* OP50 (2.8 mg (wet)/ml, ca. 1.7×10^9 cells/ml) with the test sugar(s). *E. coli* OP50 is a uracil auxotroph whose growth is largely limited in S liquid medium, which contains no organic nutrients. Control worms were incubated in the medium with no sugar added. After incubation at 20 °C for 3 days, worms were anesthetized with 25 mM sodium azide. Individual images of each worm ($n = 10$), which were selected at random, were taken with a DP70 digital CCD camera (Olympus Co., Tokyo, Japan) attached to an Olympus SMZ-9 microscope, followed by analysis using ImageJ to calculate the projection area of a worm. Each experiment was repeated two times. Statistical analysis of results were conducted by one-way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparisons test using GraphPad Prism ver. 6 (GraphPad Software, San Diego, USA). IC_{50} values were also estimated with GraphPad Prism software by nonlinear regression. The values were calculated as the concentrations needed to inhibit growth by 50% relative to controls. The axenic assay was performed in the same way as the monoxenic assay except for the use of CeMM[10,11] as a medium without *E. coli*, and the incubation period was 7 days. The original CeMM contains 180 mM D-Glc as an energy source, but we changed the concentration of D-Glc to 90 mM to avoid osmotic toxicity when a sugar was added.

We first compared the growth inhibitory effects of all the aldopentose stereoisomers in the *C. elegans* model under monoxenic culture conditions. Each stereoisomer was evaluated at a concentration of 42 mM with the growth assay using first-stage larvae (L1). Body sizes (mean area of a worm's projections ± SD) of animals treated with each sugar are shown in Figure 2. L1 larvae (body size, $0.405 \times 10^4 \pm 1220$ µm^2) were incubated for 3 days in S liquid medium with *E. coli* food and without any sugar to give egg-bearing mature adults ($5.73 \times 10^4 \pm 2980$ µm^2), which were used as untreated controls. Among the eight aldopentose stereoisomers, D-Ara showed particularly potent inhibition of nematode growth (Fig. 2). Treatment with D-Ara gave relative body sizes of 7.8% ($0.446 \times 10^4 \pm 1430$ µm^2) as compared with untreated controls. The body sizes and shapes of the D-Ara-treated animals were the nearly same as those of L1 larvae. Bright field images of typical *C. elegans* treated with 42 mM D-Ara, a control animal and an L1 larva, are shown in Figure 3. The motility of the D-Ara-treated animals apparently decreased as compared with controls, but no deaths were observed in the animals during the test period. No significant differences were observed in the concentrations of *E. coli* in the medium containing 42 mM D-Ara before and after the test period (data not shown), which ruled out the possibility that the growth inhibition was caused by a decrease in the concentration of *E. coli* food. The inhibition by D-Ara was not due to osmotic effects; when D-arabitol was substituted for D-Ara, no significant effects on growth were observed at a concentration of 42 mM ($5.25 \times 10^4 \pm 4110$ µm^2). The other seven stereoisomers at concentrations of 42 mM did not affect growth to a statistically significant extent (Fig. 2). These animals grew up to be mature adults during the test period.

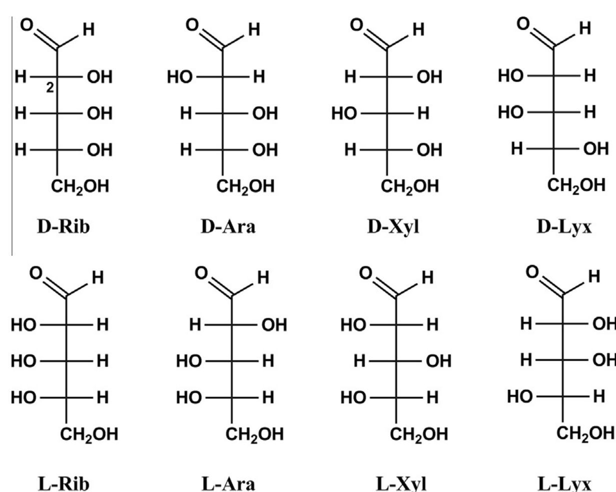

Figure 1. Chemical structures of aldopentose stereoisomers (Fischer projection). Rib, ribose; Ara, arabinose; Xyl, xylose; and Lyx, lyxose.

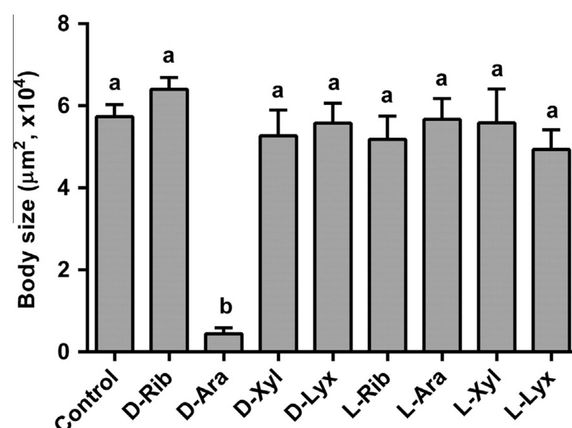

Figure 2. Growth inhibition of *C. elegans* by aldopentoses. Body sizes (mean area of a worm's projections) of *C. elegans* exposed to each sugar at a concentration of 42 mM are shown ($n = 20$ each). Error bars represent SD. Statistical difference ($p < 0.05$) is indicated by different letters. Abbreviations are as in Figure 1.

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