Activities of D- and L-Xyloascorbic Acid and D- and L-Araboascorbic Acid as a Cofactor for Dopamine β-Hydroxylase Reaction

Emiko SUZUKI,1 Tadao KURATA,2 Miho SHIBATA,3 Miki MORI3 and Nobuhiko ARAKAWA3

1 Department of Human Biological Studies, 2 Institute of Environmental Science for Human Life, and 3 Department of Nutrition and Food Science, Ochanomizu University, Bunkyo-ku, Tokyo 112, Japan
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Summary L-Xyloascorbic acid (L-xylo-AsA) and its three stereoisomers, D-xyloascorbic acid (D-xylo-AsA), L-araboascorbic acid (L-arabo-AsA) and D-araboascorbic acid (D-arabo-AsA), have been considered to show some differences in vitamin C activity. In this paper the effect of L-xylo-AsA, D-xylo-AsA, L-arabo-AsA and D-arabo-AsA on the activity of dopamine β-hydroxylase was studied to clarify whether or not the structural specificities of these stereoisomers have different effects on enzyme activity. The maximum velocity (Vmax) of the hydroxylation and Km for ascorbic acid were calculated using double-reciprocal plotting. Vmax for L-xylo-AsA was estimated to be 201 nmol/min/mg protein and those of D-xylo-AsA, L-arabo-AsA and D-arabo-AsA were 157 nmol/min/mg protein, 112 nmol/min/mg protein and 194 nmol/min/mg protein, respectively. Km for L-xylo-AsA was 1.5 mM and those for D-xylo-AsA, L-arabo-AsA and D-arabo-AsA were 2.3 mM, 2.7 mM and 1.4 mM, respectively. The effect of D-arabo-AsA on the activity of dopamine β-hydroxylase was almost the same as that of L-xylo-AsA, while D-xylo-AsA and L-arabo-AsA showed smaller effects. Our results suggest that the configuration at carbon 4 might be more important than that of the hydroxyl group at carbon 5 for the development of the activity as a cofactor for dopamine β-hydroxylase reaction.

Key Words L-xyloascorbic acid, D-xyloascorbic acid, L-araboascorbic acid, D-araboascorbic acid, dopamine β-hydroxylase

L-Xyloascorbic acid (L-xylo-AsA), which is well known as vitamin C, is known to be a cofactor in some enzymic hydroxylation reactions, including the hydroxylation of peptidyl proline residues, γ-butyrobetaine, ε-N-trimethyl-L-lysine and dopamine. The function of L-xylo-AsA as a cofactor in enzymic hydroxylation is considered a major role of vitamin C in humans. Since L-xylo-AsA has two asym-
metric carbons, C4 and C5, it has three stereoisomers, d-xyloascorbic acid (d-xylo-
ASA), l-araboascorbic acid (l-arabo-AsA) and d-araboascorbic acid (d-arabo-
AsA). It has long been believed that there is a difference in vitamin C activity
among these 4 stereoisomers, and l-xylo-AsA shows the highest activity of vitamin
C (1). However, little information on the activity of the three stereoisomers as
cofactors of the enzymic hydroxylation reaction have been reported. Hutton et al
found that d-arabo-AsA and l-xylo-AsA have the same activity on the prolyl-
hydroxylase reaction (2). Kutninik et al reported that these 4 stereoisomers have
comparative activities on the prolylhydroxylase reaction (3). Levin et al reported
that there was not much difference in activity among the 3 stereoisomers, l-xylo-
AsA, d-xylo-AsA and d-arabo-AsA, as cofactors of dopamine β-hydroxylase reac-
tion (4). However, the information is not sufficient to provide conclusive evidence
of the existence of structural specificities in the 4 stereoisomers for enzymic
hydroxylation. Therefore, in this paper the effect of 4 stereoisomers, l-xylo-AsA,
d-xylo-AsA, l-arabo-AsA and d-arabo-AsA, on dopamine β-hydroxylase reaction
was studied to clarify whether or not structural specificities for the enzyme activity
actually exist.

**MATERIALS AND METHODS**

**Preparation of stereoisomers.** l-Xylo-AsA and d-arabo-AsA were obtained
commercially (Wako Pure Chemical Industries, Osaka, Japan). d-Xylo-AsA and
l-arabo-AsA were prepared by alkaline isomerization due to a change in con-
figuration at carbon 4 of d-arabo-AsA and l-xylo-AsA, respectively, according
to the method of Brenner et al (5). The purities of d-xylo-AsA and l-arabo-AsA
were confirmed by HPLC to be 99.6%.

**Preparation of chromaffin granules.** The preparation of bovine adrenomedul-
lar chromaffin granules was carried out according to the method of Foldes
et al (6). The granules were suspended in 10 mM potassium phosphate buffer (pH
7.2) and stored at −80°C before use.

**Preparation of dopamine β-hydroxylase.** The preparation of crude dopamine
β-hydroxylase from bovine adrenomedullary chromaffin granules was carried out
according to the method of Foldes et al (6). The frozen granule suspension described
above was thawed and Triton X-100 solution was added to a final concentration
of 0.1%. The suspension was stirred for 45 min under cooling in an ice-water bath,
and centrifuged at 1,100 × g for 10 min at 4°C. The supernatant was stored on ice,
and the sediment was resuspended in a small volume of 10 mM phosphate buffer
(pH 7.2) containing 0.1% Triton X-100 and centrifuged at 1,100 × g for 10 min at
4°C. The combined supernatants were centrifuged at 100,000 × g for 10 min at 4°C,
and the supernatant dialyzed against 10 mM phosphate buffer (pH 7.2) containing
10 μM CuSO4. The dialysate was used for the assay of dopamine β-hydroxylase
activity.

**Assay of dopamine β-hydroxylase activity.** Dopamine β-hydroxylase activity
was assayed by the method of Nagatsu and Udenfriend using tyramine as the substrate (7).

Assay of protein. Protein was determined by the method of Lowry et al (8) using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Figure 1 shows a double-reciprocal plot of the reaction velocity of dopamine \( \beta \)-hydroxylase-catalyzed hydroxylation versus concentrations of each AsA isomer. The maximum velocity of hydroxylation and Km were calculated using the plot. The estimated Vmax and Km values of the isomers were as follows: L-xylo-AsA, 201 nmol/min/mg protein and 1.5 mM, respectively; D-xylo-AsA, 157 nmol/min/mg protein and 2.3 mM; L-arabo-AsA, 112 nmol/min/mg protein and 2.7 mM; and D-arabo-AsA, 194 nmol/min/mg protein and 1.4 mM (Table 1).

As D-arabo-AsA showed almost similar Vmax and Km to those of L-xylo-AsA, it can be replaced with L-xylo-AsA in this enzymic reaction. This result also agrees with those described by Hutton et al (2) and Kurata et al (9), who reported that D-arabo-AsA as well as L-xylo-AsA had comparative activities as cofactors of prolyl 4-hydroxylase reaction. These results suggest the difference in the configuration of the hydroxyl group at carbon 5 does not have any significant influence on dopamine \( \beta \)-hydroxylase activity. On the other hand, our previous papers (10, 11) showed that the content of D-arabo-AsA in the tissues of guinea pigs orally administered D-arabo-AsA was much lower than that of the L-xylo-AsA in guinea pigs orally administered L-xylo-AsA, even when the dose of D-arabo-AsA was 20 times that of L-xylo-AsA. The amount of D-arabo-AsA absorbed in the small intestine of

![Fig. 1. Double-reciprocal plot of reaction velocity of dopamine \( \beta \)-hydroxylase versus ascorbic acid isomer concentrations. ■, L-xylo-AsA; ●, D-xylo-AsA; ▲, L-arabo-AsA; ○, D-arabo-AsA. Symbols represent means and vertical bars represent SE; \( n = 3-6 \).]
guinea pigs was proven to be lower than that of L-xylo-AsA (12). On the contrary, the effect of D-arabo-AsA on the activities of dopamine β-hydroxylase (4) and prolyl hydroxylase (2, 9) was the same as that of L-xylo-AsA. These results suggest that the stereochemical difference between D-arabo-AsA and L-xylo-AsA strongly influences the absorption and retention processes of AsA in tissues more than enzyme reaction, such as hydroxylase reaction.

In a previous paper (13), we reported that the enzyme activity and liver cytochrome P-450 content of guinea pigs administered 100 mg of D-arabo-AsA were almost similar to those of guinea pigs administered 5 mg of L-xylo-AsA. The activity and cytochrome P-450 content were influenced by the vitamin C level in the tissues. However, the concentrations of D-arabo-AsA in the tissues of guinea pigs administered D-arabo-AsA were much lower than those of the L-xylo-AsA in guinea pigs administered L-xylo-AsA (10). Based on the results in this study, it is believed that D-arabo-AsA taken in the guinea pig tissues was used instead of L-xylo-AsA in some enzyme reactions. Thus, the administration of 100 mg of D-arabo-AsA could maintain a level of enzyme activity similar to 5 mg of L-xylo-AsA and, could also prevent the occurrence of scurvy in guinea pigs.

The Vmax of dopamine β-hydroxylase using D-xylo-AsA or L-arabo-AsA as cofactors was lower than that of L-xylo-AsA or D-arabo-AsA, and Km in these cases was higher than that of L-xylo-AsA or D-arabo-AsA. The results show that the difference in the configuration of carbon 4, which is closer to the ene-diol group, the key functional group for the redox reaction indispensable for AsA function in biological systems, might be more effective on the activity of dopamine β-hydroxylase. Furthermore, D-xylo-AsA induced higher enzyme activity than L-arabo-AsA. L-Arabo-AsA is the C5 epimer of D-xylo-AsA, and enzyme activity was influenced by their structural differences. Although the same structural differences exist between the two C5 epimers L-xylo-AsA and D-arabo-AsA, it did not have any influence on enzyme activity. The present results clearly show that the structural differences in their side chains have a significant effect on dopamine β-hydroxylation. Thus, when the configuration at C4 is the same as L-xylo-AsA, the configuration at C5 does not have a considerable effect on the Km or Vmax of dopamine β-hydroxylase. However, when the configuration at C4 is the same as D-xylo-AsA, the configuration at C5 has a significant effect on enzyme activity.

**Table 1.** Maximum velocity of dopamine β-hydroxylase and Km for ascorbic acids.

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<thead>
<tr>
<th></th>
<th>Vmax (nmol/min/mg protein)</th>
<th>Km (mm)</th>
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<tbody>
<tr>
<td>L-XYLOASCORBIC ACID</td>
<td>201</td>
<td>1.5</td>
</tr>
<tr>
<td>D-XYLOASCORBIC ACID</td>
<td>157</td>
<td>2.3</td>
</tr>
<tr>
<td>L-ARABOASCORBIC ACID</td>
<td>112</td>
<td>2.7</td>
</tr>
<tr>
<td>D-ARABOASCORBIC ACID</td>
<td>194</td>
<td>1.4</td>
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It is well known that dopamine β-hydroxylase is a copper-containing enzyme and needs a reductant, such as AsA, for its activity as a cofactor to reduce Cu$^{2+}$ to Cu$^{+}$, which is reoxidized during the hydroxylation of substrate (14, 15). L-Xylo-AsA is believed to be the most popular reductant in vivo. Since these four stereoisomers have the same ene-diol system fixed on a coplanar γ-lactone ring, their general electro-chemical behaviors, like electron donating properties, are considered to be almost identical. Therefore, the observed differences in hydroxylase reaction might be due to a difference in the stereochemistry of their side chain moieties, including C4. Our results suggest that the configuration at carbon 4 might have some effect on the ascorbic acid molecule in terms of approach to the copper binding site near the active center of the enzyme. Since the addition of L-xylo-AsA or D-arabo-AsA showed higher enzyme activity than the addition of L-arabo-AsA or D-xylo-AsA, the differences in enzyme activity might reflect the differences in their approach to the copper binding site in the enzyme molecule.

Our results also suggest that the configuration at carbon 4 might be more important than that at carbon 5 for the AsA molecule to develop the function as a cofactor in dopamine β-hydroxylase reaction.

REFERENCES

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