Ascorbic Acid Conjugates in Phloem Exudates of Cucurbitaceae

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17

Introduction

Although the presence of L-ascorbic acid (AsA) in the phloem has been documented for many years, it is only recently that an understanding of its role in this tissue is becoming apparent. It has been demonstrated that AsA is transported from source to sink tissues via the phloem in several plant species¹. Furthermore, phloem actively metabolises AsA being both highly active in its biosynthesis² and capable of its utilisation for the detoxification of reactive oxygen species and subsequent regeneration³. In the present work, we document AsA analogues found exclusively in the phloem of Cucurbitaceae. The function of such analogues is discussed.

Materials and Methods

Materials

Cucurbitaceae fruit were obtained from a local market. 2-O-B-D-Glucopyranosyl-i -ascorbic acid (2-GlcAsA) was isolated from partially dried Lycium barabarum fruit as described below. 6-GIcAsA was synthesised from cellobiose and AsA using cellulase⁴ and purified as outlined below.

Isolation and Purification of Analogues

Cucurbitaceae phloem exudates and L. barbarum fruit were extracted into 5% HCIO /5 mM TCEP, which was then neutralised². AsA analogues were separated from neutral and strongly acidic compounds by using strong anion exchange resin and further purified by cation interaction HPLC using 8 mM formic acid as the mobile phase. Purified compounds were lyophilised and resuspended in distilled H₂O prior to structural characterisation.

Analytical Methods

Measurement of oxidation rates⁵ and free radical quenching⁶ of AsA and analogues was as previously described. Acid hydrolysis of AsA analogues and determination of sugars by HPLC was as previously described⁵.

Results

L-Ascorbic Acid Analogues are Widespread in Cucurbitaceae Phloem Exudates

HPLC traces of phloem exudates from Cucurbita pepo

(courgette) revealed five peaks with absorption spectra almost identical to that of authentic AsA which co-eluted with peak 4 (Fig. 1). In whole fruit extracts only peak 4 (Fig. 1) could be detected (data not shown).

Phloem exudates were isolated from fruit of a number of Cucurbitaceae

species and with the exception of C. maxima all contained at least one compound with

different retention characteristics but similar absorption spectra to AsA (Table 1).

Further confirmation of the similarities between the unknown compounds and AsA was obtained from their susceptibility to oxidation by ascorbate oxidase (Fig. 2).



Physicochemical Properties of the Most Abundant L-Ascorbic Acid Analogue Isolated from C. pepo

Physicochemical properties of the most abundant AsA analogue from C. pepo (Fig. 1 peak 2) were



6-GlcAsA and the C pepo analogue were all

relatively prone to oxidation under a variety of conditions (Table 2).

On the contrary, 2-GlcAsA in which the hydroxyl group at C2 next to the enediol bond is protected was almost completely resistant to



AsA, 6-GlcAsA and

C. pepo analogue also showed similar free radical quenching kinetics (Fig. 3) while the reaction of free radicals with 2-GlcAsA was much slower. Absorption spectra of AsA.

6-GlcAsA and C. pepo analogue were similar at both pH 2.0 and pH 7.6 while that of 2-GlcAsA had a significantly lower λ_{max} at both pH's (Fig. 4)



ABTS** Radical Quenching by L-As Related Analogues

C. pepo AsA Analogue is a Glucoside

Acid hydrolysis of the most abundant C. pepo analogue (peak 2, Fig. 1) gave AsA and glucose as products (Fig. 5)



Figure 5 HPLC Traces of Reaction Products of C pepo Analogue Following Acid Hydrolysis

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Conclusions

AsA analogues are widespread in the phloem of Cucurbitaceae but absent from whole tissues

Figure 2 Oxidation of *C. pepo* Phloem Exudate Compounds by Ascorbate Oxidase

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- raffinose series oligosaccharides (polymer trap theory)
- Structural characterisation of one C. pepo analogue revealed it to be an AsA glucoside
- AsA analogues may function to load AsA into Cucurbitaceae phloem

Cucurbitaceae are symplastic phloem loaders that transport