Arabidopsis 3-deoxy-d-manno-oct-2-ulosonate-8-phosphate synthase: cDNA cloning and expression analyses

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Abstract

The molecular characterization of two isoforms of 3-deoxy-d-manno-oct-2-ulosonate (KDO) -8-phosphate synthase (AtKdsA1 and AtKdsA2) from Arabidopsis is reported here. First, by isolating a full-length cDNA for AtKdsA1, it was confirmed that the deduced primary structures of AtKdsA1 and AtKdsA2 proteins were 93% identical. Functional expression and purification studies demonstrated the efficient catalytic activity of the AtKdsA1 enzyme to produce KDO-8-phosphate from phosphoenolpyruvate and D-arabinose-5-phosphate. RT-PCR and RNA-gel blot analysis revealed different expression profiles for both genes; the AtKdsA1 gene was predominantly expressed in the shoots, while the AtKdsA2 transcript accumulated to a higher level in the roots, implicating differential roles of these isoforms in planta.

Key words: Arabidopsis thaliana, 3-deoxy-d-manno-oct-2-ulosonate-8-phosphate synthase, rhamnogalacturonan II.

3-Deoxy-d-manno-oct-2-ulosonic acid (KDO), one of the 2-keto-3-deoxy sugars, is produced in gram-negative bacteria through the activity of KDO-8-phosphate synthase (KDOS; EC 4.1.2.16) catalysing the aldol condensation reaction to yield KDO-8-phosphate from phosphoenolpyruvate (PEP) and D-arabinose-5-phosphate (Levin and Racker, 1959) and constitutes a link between lipid and D-arabinose-5-phosphate. RT-PCR and RNA-gel blot analysis revealed different expression profiles for both genes; the AtKdsA1 gene was predominantly expressed in the shoots, while the AtKdsA2 transcript accumulated to a higher level in the roots, implicating differential roles of these isoforms in planta.

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respectively. Also, AtKdsA1 protein shares 43%, 44%, and 40% identity with KDOS of *E. coli*, *H. influenzae*, and *C. psittaci*, respectively.

A recombinant AtKdsA1 protein was expressed in *E. coli* as a fusion protein with the maltose-binding protein (MBP). The full-length of the AtkdsA1 cDNA was cloned into a plasmid pMAL-c2 (New England Biolabs), and an *E. coli* strain, BL21, was used as the host for the recombinant protein expression. KDOS activity was determined at 30 °C in 0.15 ml reactions containing 50 mM potassium phosphate buffer (pH 7.5), 100 mM KCl, 0.5 mM dithiothreitol, 3 mM PEP, 3 mM d-arabinose-5-phosphate, and the enzyme protein. The reaction was terminated by adding cold 10% (w/v) trichloroacetic acid and the KDO formed was determined by the periodate-thiobarbituric method (Ray, 1980). KDOS activity of the lysate of the transformed cells increased from 7.6×10¹³ units mg⁻¹ protein to 20.1×10¹⁻³ units mg⁻¹ protein after induction with isopropyl-β-D-thiogalactopyranoside (60 µM) for 6 h at 28 °C. To confirm that the recombinant protein has KDOS activity, the fusion protein was purified to homogeneity by amylose resin column chromatography according to the manufacturer’s instruction, using a column buffer of 20 mM potassium phosphate, pH 7.5, 200 mM NaCl, 1 mM EDTA, and 5 mM 2-mercaptoethanol. The purified protein was eluted with 20 mM potassium phosphate, pH 7.5, 5 mM 2-mercaptoethanol and 10 mM maltose. The purified MBP-AtKdsA1 fusion protein exhibited the efficient KDOS activity (827 µM mg⁻¹), which was as high as that from the purified carrot enzyme (495 µM mg⁻¹; Matsuura, 2002). In this experiment, the MBP domain could not be completely removed by the incubation with the Factor Xa protease (New England Biolabs). However, MBP alone, which was split from the fusion protein and purified by Mono-Q (Amersham Biosciences) column chromatography, did not show any KDOS activity, and the KDOS activity observed with the MBP-AtKdsA1 fusion protein was ascribed to the AtKdsA1 domain, confirming that AtKdsA1 is the functional homologue of bacterial KDOS. From the sequence identity of 93% at the amino acid level, it is thought that AtKdsA2 represents the KDOS isozyme in *Arabidopsis*.

With the aim of clarifying the differential physiological roles, if any, of AtKdsA1 and AtKdsA2, the tissue specificity of the transcript accumulation of the *AtkdsA1* and *AtkdsA2* genes (Fig. 1) was examined. Probe labelling, hybridization (50 °C) and detection were carried out using a DIG-High Prime DNA Labeling and Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany). Hybridization probes were prepared by PCR. Thus, the 870 bp covering the entire ORF of AtkdsA1 cDNA was amplified using the primers of F1 and R1. This *AtkdsA1* ORF probe should hybridize to both *AtkdsA1* and *AtkdsA2* transcripts, because their ORF sequence share 86% identity at the nucleotide level. On the other hand, a 550 bp fragment from the *AtkdsA1* 3'-UTR region was also amplified by RT-PCR using a primer set of 5'-TGTTGGTGTGTTCT-3' and 5'-ACTGGATACATGTAAGAAC-3' to calibrate the amounts of cDNA templates. The conditions for all PCR were arbitrary cycles consisting of 30 s at 94 °C, 30 s at 48 °C, and 40 s at 72 °C. Amplified products were separated on 1.5% agarose gel and visualized with ethidium bromide, and the specific amplification reactions were confirmed by DNA sequencing of the PCR products.

Fig. 1. Northern hybridization analysis for *AtkdsA1* and *AtkdsA2* gene expression. Twenty µg of total RNA extracted from shoots (S) and roots (R) were blotted and hybridized with either a digoxigenin-labelled cDNA fragment of *AtkdsA1* ORF or 3'-UTR. Equal loading of RNA was confirmed by staining rRNA with ethidium bromide.

Fig. 2. Tissue specific expression of *AtkdsA1* and *AtkdsA2* genes. Semi-quantitative RT-PCR analysis was performed using total RNA extracted from shoots and roots. The RNA samples were reverse-transcribed using (dT)₃₈ as a primer, and the first strand cDNAs were amplified using following primers. A set of 5'-TTTACCTCTACTGGGACATT-3' and 5'-GCATCCAACGCAAATCAACG-3' was for a 277 bp fragment of *AtkdsA1* 3'-UTR, and another set of 5'-GATTGGAATCTCAAGGG-TCCGCTAC-3' and 5'-GACATTGGATATCATCAAGGCAAC-3' was for a 165 bp fragment of *AtkdsA2* 3'-UTR. Numbers on each lane indicate the PCR cycles. A 436 bp fragment of *Arabidopsis* actin 2 (ACT2, accession number U41998) was amplified using the primers 5'-TGGTGATTGGTGTTCT-3' and 5'-ACTGACACATGTTAC-3' to calibrate the amounts of cDNA templates. The conditions for all PCR were arbitrary cycles consisting of 30 s at 94 °C, 30 s at 48 °C, and 40 s at 72 °C. Amplified products were separated on 1.5% agarose gel and visualized with ethidium bromide, and the specific amplification reactions were confirmed by DNA sequencing of the PCR products.
References


