

Endogenous Biosynthetic Precursors of (+)-Absciscic Acid. IV. Biosynthesis of ABA from [²Hn] Carotenoids by a Cell-free System from Avocado

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Abstract

The supernatant fraction (12 000 g) of a homogenate of avocado mesocarp incorporated 3-*R*-[2-¹⁴C]mevalonate (54 µCi/µmol, 0.9 µCi/5mL) into ABA and was used to examine the incorporation of carotenoids into ABA. [²H_n]carotenoids were isolated by HPLC from mustard (*Sinapis alba*) seedlings that had germinated in closed flasks in ²H₂O (55 atom%). The carotenoids were dissolved in Tween 80 and acetone (1:1 v/v; 100 µL) and added to the cell-free system final vol. 5 mL, pH 7.1, together with cofactors. After 16 h incubation in darkness the ABA was isolated by HPLC and converted into the pentafluorobenzyl ester. The samples were analysed by capillary gas-liquid chromatography and the CH₄ chemical ionisation, negative ion mass spectra recorded. Deuterium-labelled ABA was detected when [²H] carotenoids were supplied.

'Cold traps' of unlabelled carotenoids added to the cell-free system lowered the [¹⁴C]mevalonate incorporated into ABA to 33% of the control value (1195 dpm) (*trans*-viola, 33%; *trans*-neo, 32%) but with 9'-*cis*-neoxanthin the value fell to 29%. The addition of naproxen (an inhibitor of lipoxygenases) hardly increased the ¹⁴C trapped in *trans*-viola- or *trans*-neoxanthin but reduced the ¹⁴C in ABA to 17% of the control and raised the ¹⁴C trapped by the 9'-*cis*-neoxanthin by 179%. These changes support earlier suggestions that it is 9'-*cis*-neoxanthin that is cleaved to give the future ABA residue. Other metabolic inhibitors (bisulfite, AMO 1618, tungstate, CO, piperonyl butoxide) affected the incorporation of [¹⁴C]mevalonate into ABA by the cell-free system and the incorporation of [¹⁴C]mevalonolactone into ABA formed in slices of avocado fruit in a closely similar way. This provides strong support for ABA's being biosynthesised in the cell-free system by the same reactions as those by which it is made *in vivo*.

Keywords: abscisic acid, biosynthesis, carotenoids, cell-free, 9'-*cis*-neoxanthin, avocado.

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