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IDENTIFICATION OF *MYO*-INOSITOL AS A MAJOR CARBOHYDRATE IN KIWIFRUIT, *ACTINIDIA DELICIOSA*

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Key Word Index—*Actinidia deliciosa*; Actinidiaceae; kiwifruit; isomeric form of inositol; *myo*-inositol; fruit development; fruit sugars.

Abstract—Kiwifruit plants contain unusually high levels of inositol at 20% of total soluble carbohydrate in the leaves through the season, and up to 40% of total soluble carbohydrate in parts of the fruit during mid-development. Fruit inositol content falls to 1–2% at full ripeness, mainly through large increases in the sugars rather than inositol utilisation. Kiwifruit inositol was purified by Ca^{++}-complexation chromatography and was obtained as a crystalline product. It was optically inactive, gave a ^{13}C NMR pattern identical to authentic inositol, and the same reaction rates as *myo*-inositol (but not other isomers) with *myo*-inositol dehydrogenase. We conclude that inositol is present in kiwifruit as the *myo*-isomer, and discuss its possible significance in kiwifruit physiology.

INTRODUCTION

Inositol is commonly present as a minor plant constituent, 1–2% of total plant soluble carbohydrate, to the extent that it can be regarded as a normal tissue component and plant metabolite [1]. The isomeric form is seldom checked, but is presumed to be *myo*-inositol. We uncovered an unusual situation when our analyses of sugars in leaves of kiwifruit, *Actinidia deliciosa* Liang et Ferguson cv 'Hayward', showed inositol to make up more than 20% of the sugar fraction [2], a value much higher than we were obtaining with other fruits (apple, persimmon) and leaves (from about 120 species). There seem to be very few instances where such high inositol contents have been recorded for plant tissues. In nodules of soybean and pea, high inositol was accompanied by *O*-methyl inositols [3, 4]: D-*chiro*- and *myo*-isomers were both present, and were thought to be products of the bacteriods. The remaining instances all appeared to involve halophytes (seagrasses of the Zannichelliaceae and the mangrove *Aegialitis*) [5, 6, 7], and the isomers present were mainly L-*chiro*-inositol and *muco*-inositol. Kiwifruit is neither a legume nor a halophyte, being native to the mountains of Sichuan, along the Yangtse River, a habitat completely unlike the marine estuaries where halophytes grow. We therefore set out to examine the situation in kiwifruit more closely. The following studies confirm the presence of high inositol levels, and show *myo*-inositol to be the isomeric form.

RESULTS

Sugar changes in New Zealand kiwifruit

The proportion of inositol in the leaf soluble carbohydrate was essentially constant at around 20% of 9 mg/g fresh weight from the time the leaves were half opened until approaching abscission. With both female ('Hayward') and fruiting male ('M121') New Zealand fruit, the proportion of inositol in the soluble carbohydrate fraction rose steadily to reach about 30% at 90 days after pollination (the maximum being higher in the core than the pericarp), dropped to about half as the fruit reached picking maturity (around day 165), then fell below 5% in late-picked fruit [Fig. 1(A) and 1(B)]. There was also a gradient in sugar composition from one end of the fruit to the other (C. Clark, unpub. data.). When existing data for California fruit [8] were recalculated to focus on inositol behaviour [Fig. 1(C)], the pattern of change in fruit weight, soluble carbohydrate concentration and proportion of inositol was found to be the same for New Zealand and Californian fruit, other than that New Zealand fruit took about 15–20 days longer from anthesis to maturity.

Changes in inositol content during ripening

Over the latter states of maturation from the time fruit reached full size (day 140) till harvest date (day

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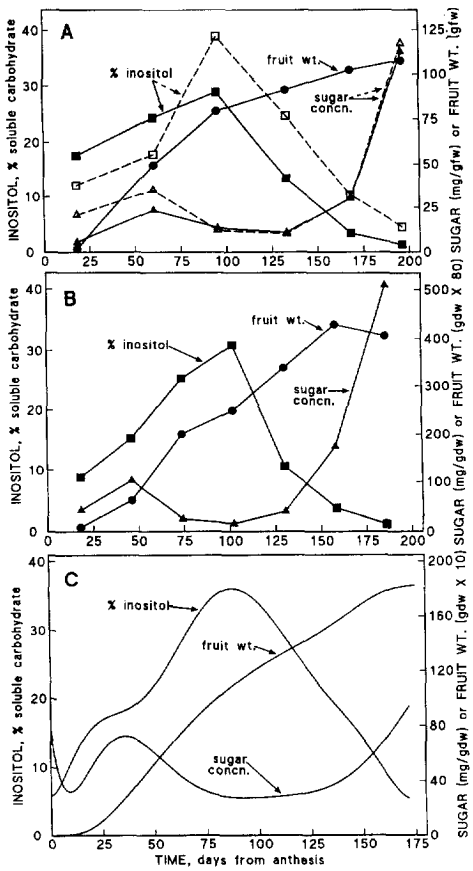

Fig. 1. Sugar concentration and relative importance of inositol in developing kiwifruit. Figure 1(A), female cv. 'Hayward' fruit (anthesis *ca.* November 27) growing under New Zealand conditions: ● fruit weight; ▲ total soluble carbohydrate concentration in outer pericarp flesh; △ total soluble carbohydrate concentration in core tissue; ■ inositol as a percentage of total soluble carbohydrate in pericarp tissue; □ inositol as a percentage of total soluble carbohydrate in core tissue. Figure 1(B), 'fruiting male cv M121' fruit: ● fruit weight; ▲ total soluble carbohydrate concentration in fruit flesh; ■ inositol as a percentage of total soluble carbohydrate in fruit flesh. Figure 1(C), female cv 'Hayward' fruit growing in California (data analysed from [8]).

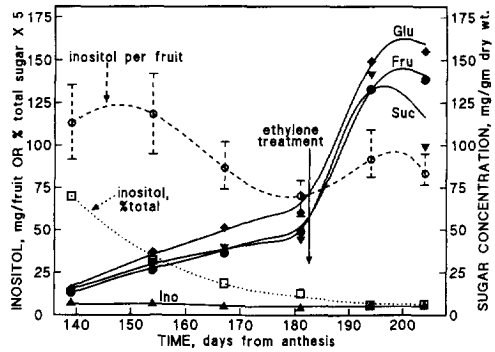

Fig. 2. Sugar changes in maturing and ripening female cv 'Hayward' kiwifruit. ▲ inositol concentration; ● fructose concentration; ▼ sucrose concentration; ◆ glucose concentration; □ inositol as a percentage of total soluble carbohydrate (sugar); ○ amount of inositol per fruit. An arrow gives the time of ethylene treatment (1000 ppm, 15 hr); bars give the s.d.

170), there was a 3-fold increase in glucose, fructose and sucrose concentration, but no change in inositol (Fig. 2). There was a further 2.5-fold increase in glucose, fructose and sucrose concentrations when ripening was triggered with ethylene, but again little or no change in inositol concentration. As a result, the proportion of inositol in the soluble carbohydrate fell from about 3.5% at harvest maturity to about 1.5% at eating ripeness. When data were used to calculate the total amount of inositol per fruit present over this time, there was found to be a very slight decrease with maturation, and an equally slight increase accompanying the large sugar increases of ripening (Fig. 2). Thus the decline in % inositol during the latter stages of fruit development was almost entirely due to increase in glucose, fructose and sucrose arising from hydrolysis of storage starch, and the absolute amount of inositol remained substantially constant.

Physical characteristics of kiwifruit inositol

A crystalline product was obtained in the expected amount from 80-day old kiwifruit using isolation methods selective for sugars and discriminatory for inositol. Comparison of the product with authentic *myo*-inositol by ^{13}C-NMR spectrum showed 4 clear singlet resonance peaks in both samples. Chemical shifts and relative intensities (brackets) were: for *myo*-inositol, δ 73.79 (99), 74.84 (100), 75.06 (51) and 77.01 (50) ppm; for kiwifruit product, 73.80 (99), 74.86 (100) 75.08 (50) and 77.02 (49) ppm, consistent with the kiwifruit product being an inositol. The compound was optically inactive ($[\alpha]_D < 0.2°$), identical to authentic *myo*-inositol, eliminating optically-active isomers (e.g. D- and L-*chiro*-inositol) as possibilities. Capillary GC of the purified product against standard *myo*-inositol and other inositol standards showed a single peak in the same position as that of inositol encountered in kiwifruit sugar extracts, both coinciding with standard *myo*-inositol and co-chromatographing with it, but being distinct from those of other isomers (retention times: kiwifruit inositol 19.91 min; *myo*-inositol 19.89; *epi*-inositol 19.19; *scyllo*-inositol 18.83; D-*chiro*-inositol 17.63; L-*chiro*-inositol 17.53).

Behaviour to myo-*inositol dehydrogenase*

The kiwifruit inositol reacted readily with *myo*-inositol dehydrogenase [Fig. 3(A)] at the same rate as authentic *myo*-inositol under a range of assay conditions for 8 assays (1.01 ± 0.04 the rate of *myo*-inositol), whereas *scyllo*-inositol, *epi*-inositol and quebrachitol (an *O*-methyl inositol) did not react (< 0.03), and pinitol reacted slowly (0.14–0.16) [Fig. 3(B)], as

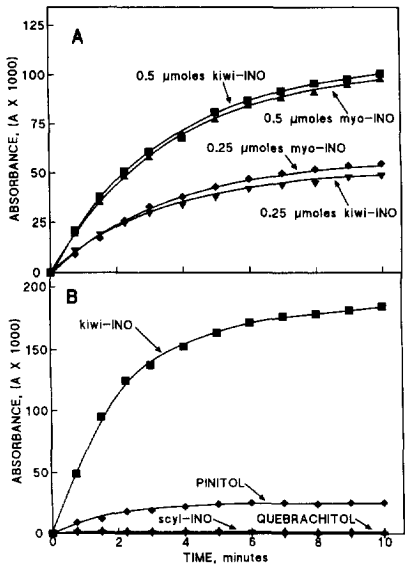

Fig. 3. Activity of kiwifruit inositol as a substrate for *myo*-inositol dehydrogenase, compared with authentic *myo*-inositol (A) and related compounds (B).

reported in [9]. We conclude that the compound present in kiwifruit is *myo*-inositol.

DISCUSSION

Earlier studies on sugars in New Zealand kiwifruit either did not record data for inositol, or were carried out on ripe fruit where inositol was a minor component at 1–2% of total soluble carbohydrate [10]. Studies on carbohydrate changes in Californian fruit during fruit development gave data on inositol, but did not calculate the contribution of inositol to total soluble carbohydrate, so that its relative importance was apparently not registered, and inositol behaviour was not commented on [8]. By recalculating the results of the California study we show that the inositol content as a proportion of soluble carbohydrate reached more than 30% at the mid-point of fruit development. We have confirmed the finding with our own analyses, and shown unambiguously that the isomer present is *myo*-inositol. *Myo*-inositol is a major component of the soluble carbohydrate fraction in kiwifruit leaves throughout their life [2], and of fruit during their development, both in the very different Californian (hot, dry) and New Zealand (mild, humid) climates, and in 'fruiting male' and female fruit (Fig. 1). Although it is not as dominant a polyol as sorbitol is in the soluble carbohydrates of apples leaves [11], its presence is comparable to that of the various *O*-methyl inositols in members of the Proteaceae (Bieleski unpub. data) and of pinitol in members of the Aizoaceae [12], Pinaceae and Leguminosae [13]. As noted earlier, there do not appear to be any published records of *myo*-inositol being equally dominant in other plant tissues, other than in water-stressed *Pinus pinaster* [13], even though its hexaphosphate phytic acid is known as a major storage form of phosphorus in storage organs [1]. Instead, the only comparable situation is to be found in functional halophytes where other isomers are involved [5–7].

We presently have only limited information on the metabolic activity of inositol in different parts of the kiwifruit plant throughout the year. Inositol can clearly be synthesised to quite high levels, but may not always be turning over very rapidly. The results suggest inositol is relatively inert late in fruit development (Fig. 2), though a lack of quantitative change does not completely rule out rapid turnover. Through lack of comparable situations where inositol is an important metabolite, we have to look at the behaviour of other polyols. In apple, sorbitol (which comprises about 60% of leaf soluble carbohydrate) is metabolically active, and plays a major part in carbohydrate transactions of the plant [11]. Fragmentary evidence suggests that inositol-based polyols, the cyclitols, may be much less actively involved in day-to-day cellular processes of the plants than sugar-based ones (mannitol and sorbitol particularly; see [11]); but on the other hand, evidence is accumulating that they can have a special role when plants encounter salt or drought stress [13, 14]. Initial results (Klages, Smith and Boldingh, unpub. data) suggest that kiwifruit plants do respond to increasing salt stress by increasing their inositol content, so salt or drought tolerance is one possible physiological function that has led to evolution of high inositol concentrations in kiwifruit. Leaf inositol could help the large mesomorphic leaves of what is basically a forest liana to resist desiccating conditions of the noonday sun in unshaded sites (such as the forest canopy or an orchard).

An alternative possibility is suggested by the role of inositol as a direct precursor of UDP-glucuronic acid on the pathway towards synthesis of cell-wall materials, particularly the polyglucuronic and polygalacturonic acids [15]. Kiwifruit plants contain about 5% of their dry weight in the form of a glucuronomannan gum having glucuronic acid as an important structural element [16]. This gum may well be synthesised in the same way, and the high inositol level could then be a consequence of the high gum content of the plant. Future research will study the rate of formation, transport and utilization of inositol relative to the sugars throughout the various stages of plant development, and attempt to learn something of its physiological significance.

EXPERIMENTAL

Analysis of tissue sugars. Leaf tissues were sampled and extracted as in [12]. Fruit from 'Hayward' female plants and 'M121' fruiting male plants, growing under standard cultural conditions for the crop on the Institute's Research Orchard at Te Puke, were harvested at various times after hand pollination (10 fruit per replicate and 3 replicates per sampling time). Female

fruit was separated into tissue zones which were weighed, lyophilised and reweighed to give dry wt. Male fruit was sliced, subsampled and lyophilised as above. Lyophilised samples were milled in a small electric coffee grinder and stored in an airtight container at −20°. Samples of 0.20 g dry wt were processed to give a sugar fr. substantially as in [12]. The method involved extraction of soluble metabolites twice in 20 ml then 10 ml of MCW (monophasic MeOH–$CHCl_3$–H_2O, 12:5:4), recovery of H_2O-soluble compounds in an aq. phase, and removal of ionic material on Sephadex SP then Sephadex QAE to yield a sugar fr. Carbohydrates were converted to TMSi derivatives (*ca.* 150 μg sugar + 80 μl Pierce Tri-Sil Z per vial, 70°, 25 min) then sepd and quantified by capillary GC on a 15 m × 0.32 mm WCOT cyanopropylphenyldimethylpolysiloxane column (J & W Scientific DB-1701) using no splitter, 1 μl injection, H_2 carrier gas at 2.0 ml min^{-1}, injector temp 250°, detector temp 260°, single FID. Running conditions were: initial temp 170° for 2 min; ramp at 1° min^{-1} to 183°; ramp at 15° min^{-1} to 250°; final temp 250° for 12.5 min.

Study of ripening female fruit. 'Hayward' fruit (5 fruit per replicate, 3 replicates per time point) was harvested at 4 times during late development. The fruit in each sample was peeled, sliced and subsampled to give a 40.0 g fr. wt sample (combined pulp and core) which was lyophilised and processed to give a sugar sample as above. Three sets of fruit were taken at the last sample time to additionally provide for two postharvest samples. These two were treated with 1000 ppm ethylene (15 hr) at 4 day after harvest to trigger ripening processes, then held at room temp. Sample 5 was taken after 8 days of ripening, when the fruit was soft, aromatic and at normal eating ripeness; sample 6 was taken 10 days later when the fruit was overripe and approaching senescent breakdown. Processing was as for the first 4 samples

Preparation of purified inositol from kiwifruit. 'Hayward' fruit (0.5 kg fr. wt) was harvested 80 days after pollination when inositol content was at a maximum, then sliced and extracted in MCW by a bulk-up of the analytical procedure [12]. Material in the aq. phase (18.37 g) was deionized to yield a sugar fraction (5.11 g of a thick syrup). This was subjected to Ca^{2+} complexation chromatography for separation of sugars [17] on 240 ml Dowex 50-W-X4 Ca^{2+} (2.5 × 42 cm column) with 10% *iso*-PrOH as solvent, and 1.25 g product per run (cf. 12). Separation was monitored by TLC [12]. Blue dextran standard emerged as a sharp peak at 0.34 × bed volume (bv), sucrose at 0.54 bv, glucose at 0.63 bv, while inositol and fructose overlapped at 0.85 and 0.86 bv. Frs containing inositol plus fructose from the 4 runs were combined, dried (1.26 g semicrystalline product), redissolved in 20 ml H_2O, and subjected to $NaBH_4$ reduction (1.0 g $NaBH_4$ 3 hr at room temp.) to convert fructose to mannitol + sorbitol. Excess BH_4^- was destroyed by adding HOAc (1 ml). Free HOAc and HBO_3 were removed by rotary film evapn from a MeOH slurry (6 ×), Na^+ was removed by ion-exchange (Dowex-50-W-X4 H^+, 30 ml column), and residual HOAc and HBO_3 were removed by MeOH evapn as before (1.01 g semicrystalline product). The product was rechromatographed by Ca^{2+}-complexation (2 runs). Inositol emerged as a sharp peak at 0.85 bv, mannitol at 1.13 bv and sorbitol at 1.54 bv. The combined inositol fraction was dried (0.58 g) and crystallized from 85% EtOH (0.44 g).

Physical inspection of the inositol. Samples of the inositol were examined by ^{13}C NMR (Bruker AM400, 100.614 MHz, in D_2O, against TSPSA reference standard [3-(trimethylsilyl)-l-propane sulphonic acid sodium salt]), in 0.5 ml solvent in a 5 mm NMR tube, proton gated decoupling, delay = 5 sec, 100–125 scans per run. Optical rotation of a sample was examined in 1% H_2O soln in a Perkin–Elmer 341 polarimeter (10 cm cell). Samples of the purified product were examined after silylation by capillary GC against the various inositol standards using the same conditions as for quantitation.

Enzymic testing of the inositol. *Myo*-inositol dehydrogenase was used to compare enzymatic behaviour of the kiwifruit product with authentic *myo*-inositol, *scyllo*-inositol, *epi*-inositol, quebrachitol and pinitol as substrates in a *myo*-inositol assay according to [9]. Each incubation mixture contained, per 1 ml cuvette sample, 10 μmol pyrophosphate buffer (pH 9.0), 0.5 μmol NADH plus 0.04 units of *myo*-inositol dehydrogenase (Sigma). Substrates added to the incubation mixture included 0.25 or 0.50 μmol kiwifruit inositol [Fig. 3(A)], or 1 μmol kiwifruit inositol, 1 μmol quebrachitol, 1 μmol pinitol or 1 μmol *scyllo*-inositol [Fig 3(B)]. Change in absorbance (340 nm, 18°) was measured over 10 min.

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REFERENCES

1. Loewus, F. A. and Dickinson, D. B., in *Encyclopaedia of Plant Physiology. Plant Carbohydrates I*, Vol. 13A, ed. F. A. Loewus and W. Tanner. Springer, Berlin, 1982, p. 480.
2. Klages, K. U., Smith, G. S. and Bieleski, R. I., *Acta Horticulturae*, 1997, **444**.
3. Skøt, L. and Egsgaard, H., *Planta*, 1984, **161**, 32.
4. Streeter, J. G., *Plant Physiology*, 1987, **85**, 768.
5. Drew, E. A., *New Phytology*, 1978, **81**, 249.
6. Drew, E. A., *Aquatic Botany*, 1983, **15**, 387.

7. Popp, M., *Zeitschrift Pflanzenphysiologie*, 1984, **113**, 411.
8. Walton, E. F. and De Jong, T. M., *Annals Botany*, 1990, **66**, 285.
9. Weissbach, A., *Biochimica Biophysica Acta*, 1958, **27**, 608.
10. Paterson, V. J., MacRae, E. A. and Young, H., *Journal of Food Science and Agriculture* 1991, **57**, 235.
11. Bieleski, R. L., in: *Encyclopaedia of Plant Physiology. Plant Carbohydrates I*, Vol. 13A, ed. F. A. Loewus and W. Tanner. Springer, Berlin, 1982, p. 158.
12. Bieleski, R. L., *New Zealand Journal of Botany*, 1994, **32**, 73.
13. Nguyen, A. and Lamant, A., *Phytochemistry*, 1988, **27**, 3423.
14. Vernon, D. M., Tarczinski, M. C., Jensen, R. G. and Bohnert, H. J., *Plant Journal*, 1993, **4**, 199.
15. Loewus, F. A., Everard, J. D. and Young, K. A., in *Inositol Metabolism in Plants*. Wiley, 1990, p. 21.
16. Redgwell, R. J., *Phytochemistry*, 1983, **22**, 951.
17. Angyal, S. J., Bethell, G. S. and Beveridge, R. J., *Carbohydrate Research*, 1979, **73**, 9.