SHORT COMMUNICATION

Effect of Temperature and Storage Time on Fructosyltransferase Activities (1-FFT and 6G-FFT) in Onion Bulb Tissues


Introduction

About 80% of onion bulb dry matter is non-structural carbohydrates (Darbyshire & Henry, 1981). These carbohydrates are predominantly glucose, fructose, sucrose and low-molecular-weight fructans, while starch and raffinose are absent (Darbyshire & Henry, 1981; Benkeblia et al., 2002). The metabolism of sugars is closely linked to the dormancy and sprouting state (Kato, 1966), and the most important biochemical change occurring during post-harvest life of bulbs, as with other vegetables, is the content variation in the carbohydrate constituents. Sprouting is characterized by a catabolism of the fructans reserve. However, an activity of fructan:fructan fructosyltransferase (1-FFT, EC 2.4.1.100) involved in fructan biosynthesis is also observed simultaneously. Nevertheless, and because the vacuole is also the site of fructan synthesis, it has been suggested that net fructan accumulation only occurs when the rate of synthesis exceeds the rate of degradation (Wagner et al., 1986). On the basis of studies of inulin formation in Jerusalem artichoke (Helianthus tuberosus), the chain elongation of fructans is mediated by 1-FFT using 1-kestose or higher β-2, 1-linked fructans as fructosyl donors and 1-kestose or higher β-2, 1-linked fructans as fructosyl acceptors to build gradually longer fructans. Neokestose is a specific trisaccharide found in Liliaceae such as onion bulb and asparagus root (Chatterton et al., 1990; Shiomi et al., 1976). Neokestose is thought to be formed by another fructosyltransferase: fructan:fructan 6G-fructosyltransferase (6G-FFT) activity using preferentially 1-kestose as fructosyl donor and sucrose as acceptor (Shiomi, 1981). 1-FFT and 6G-FFT activities during growing of onion bulbs were not extensively investigated except in the studies of Darbyshire & Henry (1979), Henry & Darbyshire (1980) and Shiomi (1989), while surprisingly no investigation was carried out on these activities during post-harvest life of onion bulbs.

The purpose of this study was to investigate the activities of the 1-FFT and particularly 6G-FFT in onion bulbs, including the effect of temperature and dormancy state.

Materials and methods

Onion bulbs

Onion bulbs Allium cepa cv. Tenshin, which had been freshly harvested and dried in the field for two weeks were obtained from the local farm of the university. They were sorted for uniformity and absence of defects, packed in commercial plastic (PVC) trays of 12 kg each. Four trays each were kept under different temperatures.

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Storage conditions

Onions bulbs were stored in the dark at two temperatures. Onions were placed in a refrigerated chamber (Eyelatron, model FLI 301 NH, Rikakikai Co. Ltd, Tokyo) at 10 ± 0.1°C and 65 ± 1% relative humidity (RH), or at 20°C in a ventilated room with an average of 50% RH.

Enzyme extraction

Enzyme extraction was conducted by the method of Van den Ende et al. (1998) and all operations were carried out on ice. Tissues (20 g) were homogenized in 40 ml of ice-cold 50 mM Na-acetate buffer pH 5 containing 10 mM NaHSO₃, 1 mM PMSF, 5 mM mercaptoethanol and 10 mM mannitol as internal standard, using a blender at full speed for two 30-second intervals. The homogenate was squeezed through three layers of cheesecloth and centrifuged at 15,000 × g for 15 min. The supernatant was then collected, and filled to 40 ml with the extraction buffer. An aliquot of 10 ml was concentrated to 1 ml by centrifugation (3000 × g for 1 h) using an Amicon Centriprep YM 10 filter (Amicon Bioseparation, Millipore, Bedford, MA, USA). The concentrate was diluted to 10 ml with the extraction buffer, then re-concentrated as above, and this process was repeated four times to remove all endogenous substrate. This enzyme extract was used for enzyme assays, sufficient to remove all endogenous substrate.

Enzyme assays

The enzymatic reactions were carried out in a total volume of 0.4 ml sodium acetate buffer pH 5 containing 0.02% (w/v) Na-azide. Activities of 1-FFT and 6G-FFT were assayed by incubating an aliquot of enzymatic extract (0.1 ml) in 50 mM sodium acetate buffer (0.1 ml) and substrate solution (0.2 ml) containing 200 mM 1-kestose (purity: 99.9%, obtained in the laboratory). The reaction mixture was incubated at 30°C for 2 h, and stopped by heating in a boiling bath for 5 min. The amount of nystose or 1⁵F, 6⁶-di-β-D-fructofuranosylsucrose formed was quantified by high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA) as described previously (Shiomi et al., 1997). One unit of 1-FFT or 6G-FFT is defined as the amount of enzyme which produces 1 μmol of nystose or 1⁵F, 6⁶-di-β-D-fructofuranosylsucrose per min under the assay conditions. 1-FFT and 6G-FFT activities were run in triplicate and expressed in nkat per g fresh weight (nkat g⁻¹).

Results and discussion

As illustrated in Fig. 1, 1-FFT and 6G-FFT activities are clearly apparent confirming the results of Shiomi et al. (1997) who reported that nystose and 1⁵F, 6⁶-di-β-D-fructofuranosylsucrose are formed by onion enzyme preparation using 1-kestose as fructosyl donor and acceptor.

During storage, 1-FFT activity increased progressively from 0.06 to 0.96 nkat g⁻¹ FW at 10°C during the first 14 weeks despite a slight decrease observed after 8 weeks. After 16 weeks, 1-FFT decreased slowly to 0.38 nkat g⁻¹ FW (Fig. 2). At 20°C, 1-FFT activity increased progressively during the first two months from 0.06 to 0.42 nkat g⁻¹ FW, and then decreased randomly to 0.08 nkat g⁻¹. During the last two months, a second and similar pattern was observed, 1-FFT activity increased to 0.48 nkat g⁻¹, then
decreased to 0.16 nkat g\(^{-1}\) after six months. On the other hand, 6G-FFT activity showed a similar pattern, although more regular than that observed with 1-FFT (Fig. 3). 6G-FFT activity increased progressively from 0.07 to 1.1 and 0.6 nkat g\(^{-1}\) FW at 10 and 20\(^\circ\)C respectively during the first four months, while the activity during the last two months remained stable ranging between 0.68 and 0.74 nkat g\(^{-1}\) at 10\(^\circ\)C, and 0.32 and 0.44 nkat g\(^{-1}\) at 20\(^\circ\)C.

It was also noted that the activities of 1-FFT and 6G-FFT were slightly higher at 10\(^\circ\)C than at 20\(^\circ\)C particularly during the last three months. This slightly higher activity observed at 10\(^\circ\)C could be induced by the availability of the substrate, a result of the degradation of fructans which is more favored by low temperature.

No investigations are referenced on 1-FFT and 6G-FFT activities in onion bulbs, or in other vegetables, during their post-harvest life. However, the synthesis rate of these two activities is low compared to the degradation rate of the enzymes involved in the catabolism of fructans particularly 1-FEH (1-fructan exohydrolase) (unpublished data). The increase of these synthesizing activities coincided with the onset of sprouting and emergence of the first sprouts from the bulb neck (unpublished data). Furthermore, it appears pertinent to note, according to Ritsema et al. (2003) and Onodera et al. (1998), 1-FF and 6G-FF saccharides are the products of a unique enzyme i.e. 6G-FFT. Nevertheless, despite the physiological stage of the bulbs characterized by a catalytic metabolism devoted to the sprouting development, it seems clear that a carbohydrates synthesis activity occurs in bulbs during this post-harvest stage. These activities probably play a role of balance among the different fructans, avoiding in this way an excessive accumulation of certain low polymerized fructans which can create a high osmotic pressure among the different scales of the bulbs. However, further investigations are needed to determine the different enzyme activities and carbohydrate status in the different parts of the bulbs (stem and scales). Purification and properties of these enzymes are also needed.

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References


