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Short communication

On the presence of starch bound phosphate in potato leaf starch

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Abstract

The presence of starch-bound phosphate in potato leaves collected late afternoon in the middle of July when the starch content is high (12.9% dry matter basis) was studied. Starch was extracted from the leaves with dimethylsulphoxide and fragments of starch were purified by ultrafiltration in two steps in combination with an α-amylase hydrolysis. The fragments were analysed with ^{31}P-NMR and no signals corresponding to phosphate monoesters linked to glucose at the C_3 and/or C_6 positions were detected. The results show that starch in potato leaves does not contain any detectable amounts of phosphate monoesters.

Keywords: Leaf starch; Phosphate monoesters; Starch extraction; ^{31}P-NMR; Ultrafiltration

1. Introduction

Native potato tuber starch contains phosphate monoesters that are mainly located in the amylopectin (Jane, Kasemsuwan, & Chen, 1996). Takeda and Hizukuri (1982) have shown that this amylopectin contains one phosphate monoester group per 317 glucose residues. The distribution of phosphate monoesters on the C_2, C_3 and C_6 positions of the glucose units of the amylopectin has been reported to be 1, 38 and 61%, respectively (Hizukuri, Tabata, & Nikuni, 1970; Tabata & Hizukuri, 1971). The phosphate groups are mainly located on the longer unit chains, 30–100 glucose units (Takeda & Hizukuri, 1982). A unit chain may contain one or more phosphate groups but no phosphate groups are located on the non-reducing end (Takeda & Hizukuri, 1981, 1982). The phosphate groups are often located close to the branch point (Frigård, 2002). The enzyme α-glucan water dikinase (GWD) is responsible for the phosphorylation at the C_3 and C_6 positions of the glucose residues in amylopectin. It is believed that amylose is not an efficient acceptor of phosphate groups (Ritte et al., 2002).

According to Bieleski (1968), over 70% of the total P in plant leaves is in the form of inorganic P, with the remainder being in the form of ribonucleic acids, phospholipids and acid-soluble phosphate esters (Ravindran, Ravindran, & Sivalogan, 1994). Small amounts of starch-bound phosphate (glucose-6 phosphate) have previously been detected in potato leaf starch (Blennow, Engelsen, Munck, & Møller, 2000). The aim of the present work was to further characterise the starch-bound phosphates in potato leaves by ^{31}P-NMR spectroscopy.

2. Materials and methods

Potatoes of the cultivar Producent were grown south of Uppsala, Sweden, and leaves were collected late afternoon in the middle of July when the starch content is high, 12.9% on a dry matter basis, (Santacruz, Koch, Andersson, & Åman, 2004). The leaves were transported in a cooling bag and freeze-dried. Amylopectin starch from potato tubers was supplied by Lyckeby Stärkelsen AB (Kristianstad, Sweden). α-Amylase from *B. amyloliquefaciens* [(1→4)-α-D-glucan glucanohydrolase; EC 3.2.1.1, 10 U/mg] was purchased from Boehringer–Mannheim (Mannheim, Germany).

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2.1. Starch extraction

Starch was extracted from potato leaves according to Santacruz et al. (2004). Disintegrated freeze-dried leaves were freed from chlorophyll and other soluble compounds, and enzymes were inactivated by boiling in 90% ethanol. After several washing steps, the remaining material was mixed with ethylenediaminetetra-acetic acid (EDTA) and stirred overnight at room temperature. After centrifugation, the starch was extracted from the pellet with 90% dimethylsulphoxide (DMSO) during heating in boiling water and in an oven. After centrifugation, the supernatants of several extractions with DMSO were combined and mixed with water to obtain a 50% DMSO solution. The solution was filtered in a stirred ultrafiltration cell (model 8400, Amicon Corp., Danvers, MA 01923, USA) with a regenerated cellulose membrane (YM3, diameter 76 mm, Amicon Corp., Danvers, MA 01923, USA) able to retain components with a molecular weight higher than 3000 Da. The filtrate was discarded, while the retained fraction (approximately 50 ml) was stirred with 300 ml of water and filtered again. After two such washing steps, the retained fraction was mixed with 99.5% ethanol (1:9 v/v). The solution was refrigerated overnight and centrifuged (1000*g* for 10 min). The starch-containing precipitate was mixed with 5 ml of water and boiled for 15 min before addition of 5 ml of 0.1 M Na acetate buffer (pH 5.0) and 0.05 mg α-amylase (Frigård, 2002). The sample was then incubated at 30 °C for 60 min and boiled for 10 min to inactivate the enzyme. After this enzymatic treatment the hydrolysed sample was mixed with approximately 20 ml of water and again ultrafiltered. The retained fraction was mixed with water and ultrafiltered twice more. The filtrates were combined and prepared for NMR analyses by repeated freeze-drying/dissolving with D_2O at least three times.

2.2. Nuclear magnetic resonance spectroscopy

The freeze-dried samples were dissolved in D_2O at a concentration of approximately 200 mg starch/ml. The samples were transferred into 5 mm NMR tubes and the pD adjusted to 8 with NaOD. Spectra were recorded on a Bruker DRX 400 spectrometer at 400 MHz for ^{1}H and 162 MHz for ^{31}P with the temperature regulated at 30 °C. Between 1000 and 20,000 transients were used. For 2D-spectroscopy, ^{1}H-^{31}P HMQC was recorded with a constant optimized for a coupling constant of 6.5 Hz. The number of scan was 256 over 128 increments.

3. Results and discussion

Starch was extracted from potato leaves with DMSO (Santacruz et al., 2004). Analysis of this relatively crude fraction with ^{31}P-NMR revealed several phosphorus signals (data not shown). Signals corresponding to starch-bound phosphate could not be identified because of overlapping with signals of other phosphorus-containing compounds. The material was therefore further purified by ultrafiltration in two steps. In the first step low molecular weight compounds, which may contain phosphorus, were removed. The retained starch was thereafter degraded with α-amylase and filtered again. High molecular weight polymers which also may contain phosphorus were retained on the filter and the degraded starch obtained in the filtrate.

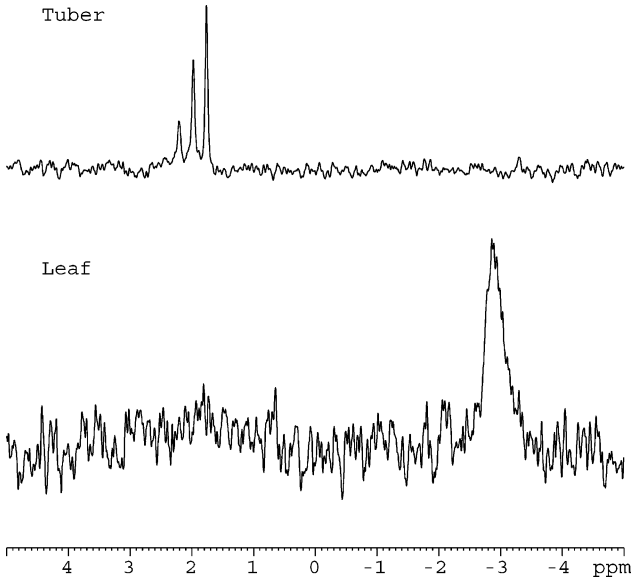

Fig. 1. 400-MHz ^{31}P-NMR spectra of partly purified starch fragments from potato tuber amylopectin starch and from potato leaf stach, both recorded at 30 °C, pD 8, in D_2O.

For comparison, potato tuber starch was degraded and filtered as described previously and showed three signals at chemical shifts of 2.21, 1.98 and 1.76 ppm in ^{31}P-NMR (Fig. 1). Both upfield signals (1.76 and 1.98 ppm) corresponded to the C_6 positions, whereas the signal at 2.21 ppm was assigned to C_3-bound phosphate as described previously by Frigård (2002). A low amylose tuber starch was chosen to avoid retrogradation of the amylose fraction, which may lead to retention of starch during the ultrafiltration process. However, the purified leaf starch sample showed no signals corresponding to starch-bound phosphate (Fig. 1). One broad signal at a chemical shift of −3.0 ppm was observed. The signal could be attributed to a lecithin carrying phosphodiester linkages (Kasemsuwan & Jane, 1995). Moreover, 2D spectra showed no cross-peaks that could reveal the presence of any phosphates linked to starch (data not shown).

The fact that starch-bound phosphate could be clearly identified in the tuber starch while undetectable in the leaf starch, even though both extracts prepared for NMR-analysis had approximately the same starch concentration, indicated that phosphate monoesters are absent or present at very low levels in the potato leaf starch.

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