The control of bud dormancy in potato tubers. Measurement of the seasonal pattern of changing concentrations of zeatin-cytokinins

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Abstract. A radioimmunoassay, combined with high-performance liquid chromatography, has been used to analyse the zeatin-type cytokinins of potato (Solanum tuberosum L. cv. Majestic) tubers and tuber buds throughout growth and storage. During tuber growth, zeatin riboside was the predominant cytokinin detected in all tissues. Immediately after harvest, the total cytokinin concentration fell dramatically in the storage tissue, largely as a consequence of the disappearance of zeatin riboside. During storage, levels of cytokinins in the storage tissue remained relatively constant, but increased in the tuber buds. In the buds of tubers stored at 2°C there was a 20- to 50-fold increase in total cytokinin over six weeks, coinciding with the natural break of innate dormancy. At 10°C the rise in the level of bud cytokinins was slower, correlating with the longer duration of innate dormancy. Injecting unlabelled cytokinins into tubers in amounts known to induce sprouting gave rise to increases in cytokinin concentrations in the buds of the same order as the increase associated with the natural break of dormancy. Metabolism of injected cytokinins was greater in non-dormant than in dormant tubers.

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Abbreviations: CK = cytokinin; FW = fresh weight; HPLC = high-performance liquid chromatography; RIA = radioimmunoassay; tio6ade = 6-(4-hydroxy-3-methylbut-trans-2-enylamino)-purine = zeatin; tio6adeglc9 = 6-(4-hydroxy-3-methylbut-trans-2-enylamino)-9-[3-D-glucopyranosylpurine = zeatin-9-glucoside; tio6ado = 6-(4-hydroxy-3-methylbut-trans-2-enylamino)-9-[3-D-ribofuranosylpurine = zeatin riboside; tio6ado-[H]-diol = a radioactive derivative of zeatin riboside, synthesised by periodate-oxidation followed by [PH]NaBH4-reduction; tio6AMP = 6-(4-hydroxy-3-methylbut-trans-2-enylamino)-9-[3-D-5'-phosphoribofuranosyl]purine = zeatin riboside 5'-monophosphate; tio5glic46ade = 6-(4-O-β-D-glucopyranosyl-3-methylbut-trans-2-enylamino)purine = zeatin-O-glucoside

The roles of cytokinin concentration and the sensitivity of the buds to cytokinin in the control of dormancy are discussed.

Key words: Bud dormancy (innate) -- Cytokinin metabolism -- Solanum (bud dormancy).

Introduction

In a previous paper (Turnbull and Hanke 1985), evidence was presented that treatment with cytokinin (CK) can break the dormancy of potato tuber buds. However, exogenous CK was effective only during short periods at specific stages in the potato life-cycle, following the beginning and preceding the end of innate dormancy. During the period in between, the innate dormancy of the tuber buds was not affected by exogenous CK.

To find out whether or not endogenous CK is involved in the natural control of potato bud dormancy, we first have to know the content of CKs in the buds at all stages in the life cycle of the tuber. Published measurements of the endogenous CKs of potatoes (Okazawa 1969; Van Staden 1976; Van Staden and Dimalla 1978; Koda 1982a, b) were all obtained by bioassays after separation using methods with low resolving power, e.g. paper- or Sephadex LH 20-chromatography, which limits the usefulness of the results. In tuber tissue, CK activity co-chromatographing with zeatin (tio6ade), zeatin riboside (tio6ado), zeatin riboside-5'-phosphate (tio6AMP) and isopentenyladenine was reported. Nothing at all is known about CKs in unsprouted buds.

It therefore seemed necessary to analyse and estimate the endogenous CKs of tubers and their buds and to investigate CK metabolism in these tissues. The only technique sensitive enough to
measure the CKs of unsprouted buds [approx. 100 μg fresh weight (FW) per bud] is immunoassay and so a radioimmunoassay (RIA) for CKs with a zeatin-type (tio 6) side chain on N6 was developed from the method of Weiler (1980). Wherever possible the CKs in an extract were fractionated using high-performance liquid chromatography (HPLC) (Horgan and Kramers 1979) before estimation by RIA. These analytical methods were used to follow the changing pattern of CK in various tissues, including tuber buds, at different stages in the potato life cycle.

Materials and methods

Plant material. Potato (Solanum tuberosum L. cv. Majestic) was grown, harvested and stored as described previously (Turnbull and Hanke 1985).

Chemicals. Cytokinins were from Sigma Chem. Co., Poole, Dorset, UK, except zeatin-7-glucoside, zeatin-9-glucoside (tio 4-ade glc4), dihydrozeatin-O-glucoside ([tio 4-gluc4] dih dze ade), zeatin-O-glucoside and tio 6-AMP which were generous gifts from Dr. R. Horgan (University College of Wales, Aberystwyth). All CKs were checked for purity by reverse-phase HPLC and where necessary purified before estimating cross-reactivity in the RIA.

Feeding tubers with cytokinins. Cytokinins (200-400 μM) were injected into whole tubers as described previously (Turnbull and Hanke 1985). A radioactively labelled CK derivative (tio 6-ado-[14C]-dol, see subsequent “Radioimmunoassay” section for synthesis) was used to estimate the volume of CK solution infiltrated into the tuber tissues. From the total extractable radioactivity subsequently recovered, values of 10–20 μl per tuber were calculated, corresponding to a CK dose of 2-8 nmol per tuber.

Cytokinin extraction from plant tissues. Although a methanol/CHCl3/HCOOH/H2O mixture (Bielaske 1964) is the preferred extractant for CKs, its use gave rise to severe interference in the RIA, masking CKs detectable in 80% ethanol extracts. The nature of this interference could not be determined. In consequence, 80% ethanol was used as extractant in the knowledge that this solvent does not completely inactivate phosphatases and so a proportion of any CK present as the nucleotide in the tissue might be detected as the nucleoside. The protocol for extraction was designed to minimise any such hydrolysis.

The outer cortex (2–3 mm depth of ‘peel’) of the tuber in the vicinity of the buds and the buds or sprouts themselves were used for the majority of analyses. Tissue from at least 10 tubers was taken for each extract. Bud samples were prepared by individual dissection, discarding the protective bud scale leaves to ensure that only living tissue was taken. With practice, buds could be excised at 200–300 (20–30 mg FW) per hour. The time from excision of each bud to freezing in liquid N2 was 10–20 s. Tissue samples were frozen immediately in liquid N2 and extracted without thawing in chilled (–20°C) 80% ethanol (10:1, v/w). Storage, where necessary, was at –90°C as CK losses were detected after storage at –20°C. After 1-2 h, the homogenate was centrifuged (1000 gav for 2 min in a Microfuge B (Beckman, High Wycombe, Buck., UK), or 35000 gav for 20 min in a J-22L (Beckman)). The clear supernatant, ‘crude extract’, was frozen in liquid N2 and stored at –90°C if not analysed immediately.

Factors in the crude extract which interfered in the RIA could be removed in a single step using C18 Sep Paks (Waters, Harrow, Mddx., UK). Methanol, 4 ml, followed by 10 ml of 10 mM triethylammonium acetate (TEAA) solution, pH 7, was passed through the Sep Pak cartridge before crude extract from up to 5 g FW tissue was loaded and washed through with a total of 9 ml of 10 mM TEAA solution, pH 7. Cytokinins were retained on the cartridge and could be eluted in 6 ml 40% (v/v) methanol in 10 mM TEAA solution. Afterwards 6 ml methanol was passed through to clean the cartridge. The 40%-methanol fraction was evaporated at low pressure at 35°C to <0.2 ml g–1 FW original tissue, cleared by centrifugation (9000 gav for 2 min) and either analysed by HPLC, or estimated by RIA, or frozen in liquid N2 and stored at –90°C.

High-performance liquid chromatography. The equipment, from Spectra Physics, St. Albans, Herts., UK, consisted of an SP7800 solvent delivery system coupled to an SP8300 UV(254 nm) detector and an SP4100 computing integrator. Samples were loaded using a Rheodyne 7125 injector fitted with a 1000 μl sample loop. C18-bonded silica reverse-phase columns were used; most analyses were carried out on an Ultrasphere 5 μm ODS column (150 mm long, 4,5 mm diameter; Anachem, Luton, Beds., UK). The mobile phase was a mixture of methanol (LC grade, Fisons, Loughborough, Leics., UK) and water (from a glass still fed with distilled water) containing 0.2 mM TEAA, pH 7. Solvents were vacuum-degassed and helium-purged before use. Gradient elution was sometimes used, but in practice all the CKs of interest could be separated in 10 min by an isocratic programme of 30% methanol at 1 ml –1 (Fig. 1).

Only extracts from small amounts of tissue (5–50 mg FW) were injected without prior clean-up on a C18-Sep Pak. Samples of any volume up to 0.9 ml could be loaded, made up to 1 ml with H2O. In this solvent, CKs are immobilised on the column head where they accumulate with very little loss of resolution. For preparative separations, fractions of 11 drops collected by a Redicar 2122 (LKB, Bromma, Sweden) were of equal volume. Thirty fractions were collected from 0.95 (void time) to 11 min after injection, dried in vacuo and redissolved in H2O before RIA.

A mixture of all the CKs of interest (10–200 pmol of each compound) was run immediately before and after the fractionation of an extract, and the retention times of the standards were used to identify the peaks detected by RIA in the sample.

Radioimmunoassay. Because almost all CKs reported as occurring in potatoes co-chromatographed with members of the zeatin family of compounds, an RIA method of Weiler (1980) for CKs with the tio 6-[6-(4-hydroxy-3-methylbut-trans-2-enyl)] side-chain was adapted for our use.

(a) Preparation of antigen. An immunogenic CK-conjugate was synthesised by the method of Erlanger and Beiser (1964) as described by Weiler (1980): the furanose ring of tio 6-ado is cleaved between carbons 2 and 3 by oxidation with IO4–, resulting in a dialdehyde. This reacts with the primary amino groups of bovine serum albumin to form a linkage which is stabilised by reduction with NaBH4. In our reaction, 70.9 mg conjugate was recovered which, from UV difference-spectra,