Phytochemistry, 1977, Vol. 16, pp. 309–310, Pergamon Press. Printed in England.

ADENOSINE CYCLIC MONOPHOSPHATE IN POTATO TUBERS DURING STORAGE AT +10° AND +2°

FREDERICK A. ISHERWOOD and STEPHEN G. RING

Agricultural Research Council, Food Research Institute, Colney Lane, Norwich NR4 7UA, U.K.

(*Revised received* 30 *September* 1976)

Key Word Index— *Solanum tuberosum*; Solanaceae; tuber; adenosine; cyclic monophosphate; temperature changes.

Abstract—The levels of adenosine 3′:5′ cyclic monophosphate in mature potato tubers (variety King Edward) stored at +10°, for varying periods at +2° and then after 28 days at +2° transferred to +10°, have been measured. The concentrations were very low (3–30 pmol/g fr. weight) and did not show marked changes with storage conditions.

Adenosine 3′:5′ cyclic monophosphate (cyclic AMP) is claimed to be present in higher plants and in view of its diverse physiological roles in animal tissues it was of interest to determine whether it played any part in the carbohydrate changes which occur in dormant potato tubers when they are subjected to a change in temperature of storage. At 2° starch is converted into sugar (after a lag period of 5–8 days) and if the sweetened potatoes are transferred back to +10°, the sugar is mostly converted into starch [1]. In animals cyclic AMP stimulates the conversion of inactive to active glycogen phosphorylase and thereby increases the breakdown of glycogen to glucose so it is a plausible assumption that cyclic AMP may affect carbohydrate interconversion in plants.

In the present study a method has been developed based on an earlier general method for the isolation of phosphate esters [2] from plant tissues which effectively eliminates substances other than cyclic AMP from the final purified extract. Treatment with a phosphodiesterase which only splits 3′:5′ cyclic nucleotides under standard reaction conditions left no material which reacted in the Gilman [3, 4] binding assay.

Analyses were made on samples from the physiological experiments described previously [1] and spanned conveniently the period of 'sweetening' (28 days at +2°) and 'conditioning' (28 days at +10° when most of the sugar is converted into starch).

RESULTS AND DISCUSSION

Preliminary results with whole potato tubers (var. King Edward) indicated that the level of cyclic AMP was much lower than many of the figures given in the literature (0.1–9.6 nmol/g fr. wt) would suggest [cf 5, 6]. The isolation of such a small amount of a particular nucleotide from plant tissue is difficult and in developing an extraction and purification method we were guided by previous experience in isolating trace amounts of nucleotides and phosphate esters from potato tissue by a method which was originally developed in this laboratory for the isolation of phosphate esters but has been refined and improved in detail subsequently. Particularly in the case of potato tissue, trouble is experienced because of the presence of iron in the extract, which can cause very large losses of even a simple sugar phosphate such as fructose-6-phosphate. These losses can be avoided if the iron is removed by the use of a chelating resin whenever there is reason to suspect its presence (in the original extract and after elution of nucleotides from the activated charcoal column). On such a resin the retained iron does not adsorb nucleotides and phosphate esters which then pass into the eluate.

Table 1. Cyclic AMP in whole potato tubers (var. King Edward) stored at +2 and at +10°

Storage conditions		Cyclic AMP pmol/g fr. wt Expt 1	Expt 2
+10° for 35 days		10.6	
from +10° to +2°	2 days	30	26
	14 days	11	—
	28 days	7	3
	56 days	8	7.3
after 28 days at +2° transfer to +10°	3 days	8.8	9
	10 days	8	9
	28 days	19	—

In the original method for the isolation of phosphate esters from plant material the sugars and other neutral solutes were removed from the extract by cellulose chromatography using as solvent a mixture of *n*-PrOH, water with a trace of ammonia and EDTA. Practically all the phosphate esters and nucleotides were left behind on the cellulose. However, a careful check on the eluate from the column showed that it contained the cyclic AMP from the original extract and this meant that most other nucleotides and sugar phosphates had been removed. The cyclic AMP was then freed from the sugars, amino acids and salts also present by adsorption onto charcoal and elution with aqueous ethanol containing a small amount of NH_3. The cyclic AMP was finally purified by PC using a mixture of *n*-PrOH, aq. NH_3 (18 M) and H_2O (6:3:1) as solvent. The progress of the isolation procedure was monitored by adding cyclic AMP [$U^{14}C$] to the original extract. The amount was limited to less than 30% of the total (estimated from preliminary experiments) in order not to interfere with

the accuracy of the final estimation. This required material with a high sp. act. (in the present experiments we used a sample with a sp. act. of 209 mCi/mmol). The overall recovery of the marker AMP was about 40%. The main losses occurred during the charcoal adsorption and elution step (30–35%) and to a lesser extent during the separation from other nucleotides on the cellulose column (20–25%). The final results were corrected for the [$U^{14}C$] cyclic AMP still present.

Results for potato samples stored at +2° and 10° given in Table 1 suggest that there is a small change when potato tubers are first transferred to +2° but the significance is difficult to evaluate. At 14 days when starch is rapidly converted into sugar the figure is very close to the control sample. Transfer back to +10° after 28 days at +2° (the potatoes contain about 2·5% total sugar) causes a rapid loss of sugar but very little change in the cyclic AMP. The level of cyclic AMP is in general very low confirming the results of other workers [5], and it seems unlikely that it is acting as an amplifying agent for plant hormones [6]. The possibility that it might be due to surface contamination by micro-organisms was considered but seemed unlikely because the potato tubers were carefully washed before analysis.

EXPERIMENTAL

Measurement of radioactivity. Radioactivity was measured with a liquid scintillation spectrometer. All measurements were made in 2 ml of a toluene–Triton (2:1) scintillation fluid containing 4 g/l. PPO except for the Millipore filters used in the binding assay which were analysed as specified by Boehringer in a mixture of toluene and 2-methoxy ethanol containing 8 g/l. PPO. After PC radioactivity in zones was located and measured directly using a chromatogram scanner.

Extraction procedure. Potato powder (15 g) was added with stirring to a mixture containing 10% (TCA), 30% MeOH and 0.15% 8-hydroxy quinoline in H_2O (35 ml) at −40° and then blended (5 min). Homogenate was centrifuged and the residue reextracted with 5% TCA (35 ml) for 5 min and again centrifuged. Combined supernatants were shaken out with an equal vol. of a mixture of Et_2O and petrol (40–60°) (2:3) and the process repeated × 5 until the top layer was free from TCA. The aq. soln (pH 2.5) was neutralised with NH_3 and evaporated *in vacuo* to 15 ml to remove MeOH. H_2O was added (85 ml) and the soln acidified with $HC10_4$ (pH 2) and shaken out with Et_2O (150 ml, × 3) to remove traces of TCA which were present as the anion in the original extract. The aq. layer was neutralised with NH_3 (pH 8.4), Et_2O removed *in vacuo*, and the soln passed through a column (12 × 1 cm) of Chelex 100 resin (NH_4 salt, 200–400 mesh). Combined effluent and washings were then passed through a column (12 × 1 cm diam) of Dowex AG 50 W (X2, 200–400 mesh, NH_4 salt) and finally the soln was ultrafiltered (Millipore Pellicon membrane PSED 14205) and freeze-dried.

Cellulose chromatography. Residue dissolved in H_2O (0.5 ml) was mixed with cellulose powder (5 g chromedia CF1 Whatman) and made into a slurry with the solvent, a mixture of *n*-PrOH, H_2O, aq. NH_3 (18M) and 2 mM EDTA (90.3:9.5:0.1:0.1). The slurry was added to the top of a column (10 × 2 cm diam) of cellulose powder (5 g) which had been previously well washed with solvent. The column was then developed with the same solvent (250 ml) until the radioactivity of the eluate was negligible.

Charcoal adsorption. Combined eluates were evaporated to almost dryness to remove PrOH, dissolved in H_2O (100 ml) and the soln passed slowly through a column of well washed charcoal (5 × 1 cm Nuchar C190, 52–120 mesh) to adsorb the nucleotides and then the column well washed with H_2O to remove sugars and amino acids. The nucleotides were eluted with a mixture of EtOH, H_2O and aq. NH_3 (18M) (25:75:0.8, 3 × 25 ml) until the final eluate contained a negligible amount of radioactivity. The combined eluates were freeze-dried, taken up in H_2O (25 ml) and then passed through a small column (5 × 1 cm) of Chelex 100 resin as before to remove iron leached out of the charcoal.

Removal of phenols. Combined eluates (pH 3; formic acid) were passed through a column of PVP (5 × 1 cm Poly Clar AT powder) and then freeze-dried. Residue was dissolved in H_2O (0.2 ml).

Paper chromatography [7]. Solvent used was a mixture of *n*-PrOH, aq. NH_3 (18M) and H_2O (6:3:1) and the position of the cyclic AMP was located from the radioactivity of the marker cyclic AMP-[$U^{14}C$] added to the original extract. Cyclic AMP was eluted from the appropriate place and then assayed [3]. Appropriate checks were made that no interfering substances were present in the final extract, which would lead to either a high or low assay. In particular, treatment with the 3′:5′ phosphodiesterase under conditions which were adequate to destroy the adenosine 3′:5′ cyclic monophosphate left no active material behind. Cyclic AMP added to the extract gave the expected response.

Enzymes. The kit for the measurement of cyclic AMP by the binding protein method [3] was purchased from Boehringer Mannheim G mnH and the phosphodiesterase from Sigma Chemical Company, U.S.A.

REFERENCES

1. Isherwood, F. A. (1973) *Phytochemistry* **12**, 2579.
2. Isherwood, F. A. and Barrett, F. C. (1967) *Biochem. J.* **104**, 922.
3. Gilman, A. G. (1970) *Proc. Natl. Acad. Sci.* **67**, 305.
4. Brown, B. L., Albano, J. D. M., Ekins, R. P., Sgherzi, A. M. and Tampion, W. (1971) *Biochem. J.* **121** 561.
5. Bressan, R. A., Ross, C. W. and Vandepeute, J. (1976) *Plant Physiol.* **57**, 29.
6. Amrhein, N. (1974) *Planta* **118**, 241.
7. Hanes, C. S. and Isherwood, F. A. (1949) *Nature* **164**, 1107.