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CHANGES IN THE ACTIVITY OF HYDROXYCINNAMYL CoA:QUINATE HYDROXYCINNAMYL TRANSFERASE AND IN THE LEVELS OF CHLOROGENIC ACID IN POTATOES AND SWEET POTATOES STORED AT VARIOUS TEMPERATURES

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Key Word Index—*Solanum tuberosum*; Solanaceae; *Ipomoea batatas*; Convolvulaceae; potato tuber; sweet potato tuber; hydroxycinnamyl CoA: quinate hydroxycinnamyl transferase; chlorogenic acid levels; chilling injury.

Abstract—A large increase in the activity of hydroxycinnamyl CoA:quinate hydroxycinnamyl transferase (CQT) occurred in potatoes stored at 0 and 2° and such an increase was prevented by storage at either 5 or 10°. The increase was most rapid in potatoes stored at 0° where it reached a maximum after 28 days and then declined slowly during storage for up to 6 months. Accompanying these changes in CQT were transitory increases in *p*-coumarate CoA ligase and PAL which occured during the first few weeks of storage at 0° and during this period there was nearly a two fold increase in the chlorogenic acid content of the tissue. The increase in chlorogenic acid did not occur at 10° when the increases in PAL, ligase and CQT were also prevented. The increase in CQT was reversed when tubers stored at 0° for 14 days were returned to 10° and this warming up period prevented further increase in CQT on return to 0°. The increase in CQT at 0° was prevented if the air in the storage atmosphere was replaced by N_2, 1% O_2 or 10–15% CO_2. Similar increases in CQT, ligase and chlorogenic acid occurred in sweet potatoes stored at 7.5° but were prevented by storage at 15°. The role of PAL, ligase and CQT in the control of chlorogenic acid accumulation in these commodities and the significance of changes in their activities in relation to physiological changes at low temperatures are discussed.

INTRODUCTION

In a previous paper on the changes in the activity of enzymes of phenylpropanoid metabolism in tomatoes stored at various temperatures [1], it was shown that an enzyme involved in chlorogenic acid metabolism hydroxycinnamyl CoA:quinate hydroxycinnamyl transferase (CQT) [2, 3] increased markedly at low temperatures. The increase in activity occurred at temperatures below the threshold temperature (12–13°) for chilling injury in the tomato but did not occur at temperatures above this critical temperature.

In the present paper, the behaviour of the enzyme in two further plant tissues stored at low temperatures was investigated. The sweet potato has a threshold temperature for chilling injury of 10–12° [4] while the potato is generally considered to be insensitive to chilling injury [5]. However, physiological changes in the potato are known to occur at temperatures below 5° and particularly low temperature sweetening has been widely studied [6]. Other workers [7–9] have described a form of injury in the potato which occurs only at very low temperatures (below 2°). The injury leads to the formation of discoloured patches on the skin and of dark brown areas in the flesh. In the present work, in addition to changes in the level of CQT, the changes in the activity of phenylalanine ammonia lyase (PAL) and *p*-coumarate CoA ligase and in the concentration of chlorogenic acid in both sweet potato and potato were followed during storage at a range of temperatures.

RESULTS AND DISCUSSION

CQT from potatoes was purified by DEAE-cellulose chromatography by the method previously used for the tomato enzyme [3]. Table 1 shows some of the properties of the potato enzyme which catalyses both the synthesis and breakdown of chlorogenic acid. In the breakdown of *p*-coumarylquinic acid, the enzyme is specific for the 5′ isomer (IUPAC nomenclature). The enzyme fraction from DEAE has activity with shikimic acid giving 16% of the rate with quinic acid. Neither glucose nor caffeic acid is a substrate for the enzyme. The K_ms for caffeyl CoA and for 5′-*p*-coumarylquinic acid are very similar to the values obtained with the tomato CQT. However, the potato enzyme has a lower affinity for chlorogenic acid than the enzyme in tomato.

Fig. 1(a) shows that potatoes (var. Majestic), after a preliminary curing period at 10°, have a very low level of CQT activity which changes very little during storage at either 5 or 10° for up to 160 days. However, if the cured potatoes are stored at 0 or 2°, there is a major increase in activity during storage, the increase being greater at 0 than at 2°. Fig. 1(b) shows the early stages in the rise in CQT activity during storage at 0° with a lag phase of about 3 days before the rapid rise in activity which reaches a peak, eleven fold greater than the initial activity, after 28 days [Fig. 1(a)] and then decreases slowly. In these experiments the activities of PAL and *p*-coumarate CoA ligase were also followed. The levels of these two enzymes were initially low and rose to maximum levels of 0.27

Table 1. Properties of CQT prepared from potato tubers stored at 10°

	pkat/mg protein	K_m (μM)
DEGRADATION REACTION		
Chlorogenic acid + CoA	240	172
5′-*p*-coumarylquinic acid + CoA	170	118
4′-*p*-coumarylquinic acid + CoA	0	—
3′-*p*-coumarylquinic acid + CoA	0	—
SYNTHETIC REACTION		
Caffeyl CoA + quinic acid	110	34
Caffeyl CoA + shikimic acid	18	—
Caffeyl CoA + glucose	0	—
Caffeic acid + quinic acid	0	—

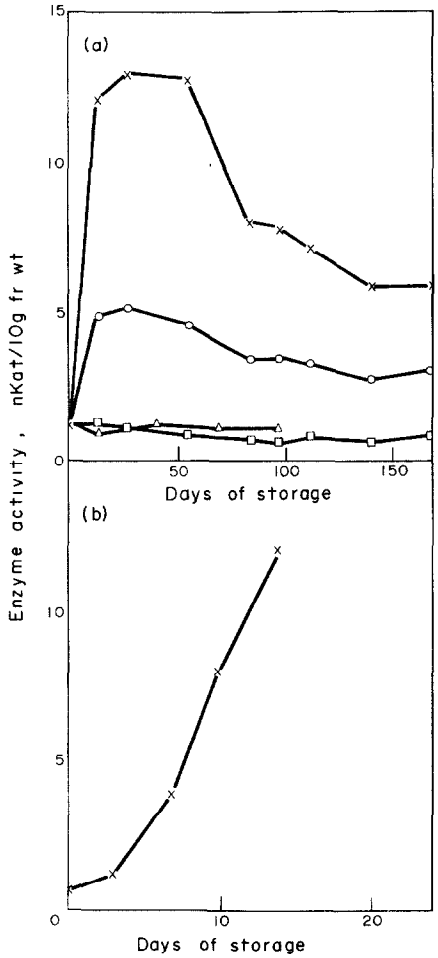

Fig. 1. Changes in the activity of CQT in potatoes (var. Majestic) stored at 0° (×——×), 2° (○——○), 5° (△——△) and 10 (□——□) for up to 24 weeks (a) or for up to 14 days (b).

and 0.53 nkat/10 g fr. wt respectively for PAL and ligase after 14 days storage at 0° and then decreased again to low levels. Smaller increases occurred during the first 14 days of storage at 2° while at 10° no increase was observed. The experiments described in this paper were carried out using potatoes of the variety Majestic. However, many other experiments have shown similar increases in CQT during low temperature storage of other varieties, including King Edward, Homeguard and Desiree.

Fig. 2(a) shows changes in the levels of chlorogenic acid in potatoes which had received the same treatment as those in Fig. 1(a). At 10° the level of chlorogenic acid is low (2.2 μmol/10 g fr. wt) and this decreases slowly during storage. At 0° there is a rise during the first 56 days to a maximum, 1.8 times greater than the initial level, and then a steady decline. At 2° the level of chlorogenic acid rises 50 % during the first 84 days of storage and then declines slowly. The extent of the increase in chlorogenic acid during storage at either 0 or 2° is considerably less than the rise in CQT activity. The rise in CQT activity seems to correlate in a general way with the accumulation of chlorogenic acid but the relationship does not appear to be very precise. As has been discussed before [3], the metabolic function of CQT is still to be defined as it can act both in the synthesis and cleavage

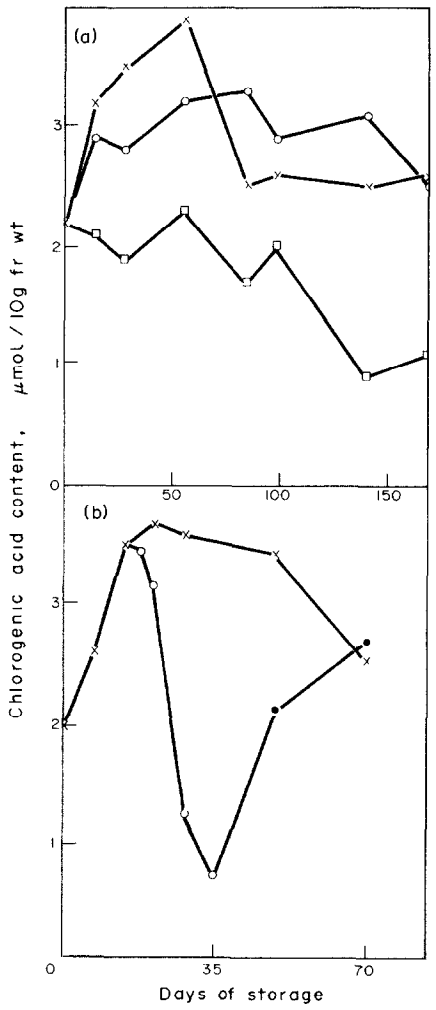

Fig. 2. (a) Changes in the concentration of chlorogenic acid in potatoes (var. Majestic) stored for up to 24 weeks at 0° (×——×), 2° (○——○) and 10° (□——□). (b) Changes in the concentration of chlorogenic acid in potatoes (var. Majestic) stored either at 0° throughout (×——×), 0° for 14 days followed by 21 days at 10° (○——○) or 14 days at 0° followed by 21 days at 10° and a further 35 days at 0° (●——●)

of chlorogenic acid. The extent to which chlorogenic acid is an end product of the pathway and the degree to which it turns over in potato tissue has yet to be determined.

Figs 2(b) and 3 show data for changes in the CQT activity and the chlorogenic acid content of potatoes initially stored for up to 70 days at 0°. This shows the rise in CQT and the accompanying increase in chlorogenic acid over the first 28 days at 0°. If potatoes were transferred from 0 to 10° after 14 days, the CQT activity, after a lag phase of two days, declines steadily to reach a value after 21 days at 10° of only 20% of that at the time of transfer. This fall in transferase is accompanied by a large fall in chlorogenic acid content [Fig. 2(b)]. If the potatoes after 21 days at 10° are again returned to 0°, there is very little increase in CQT activity but the concentration of chlorogenic acid rises steeply to a level after 35 days which is similar to that in potatoes stored at 0° throughout the experiment. This experiment shows that, although there is a general correlation between the changes in CQT activity and chlorogenic acid level during the transfers to 0° and from 0 to 10°, it is clear that in the subsequent transfer back to 0° the level of CQT activity does not control the biosynthesis of chlorogenic acid and other controlling factors must come into play.

Table 2 shows that atmospheres of N_2 and 1% O_2 completely prevent the increase in CQT which occurs when potatoes are stored at 0°. Similarly, 10 and 15% CO_2 almost completely prevent the increase while 5%CO_2 and 3–5% O_2 cause partial inhibition of the increase in activity. These results suggest that respiratory processes play some role in the rise in CQT even if this is indirect. In other results it was shown that if potatoes which had been stored at 0° for 21 days, and which had high CQT activity, were transferred to 10°, either in air or in 10% CO_2, the CO_2 atmosphere did not prevent the fall in CQT at 10°.

Fig. 4(a) shows that sweet potatoes stored at 15° for up to 98 days show a steadily declining level of CQT

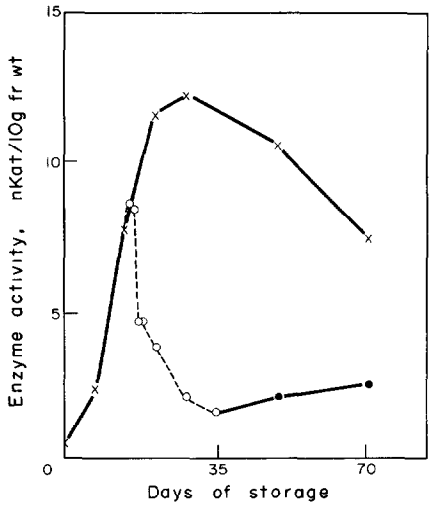

Fig. 3. Changes in the activity of CQT in potatoes (var. Majestic) which were stored either continuously at 0° for up to 70 days (×——×), or for 14 days at 0° followed by 21 days at 10° (O——O) or for 14 days at 0° followed by 21 days at 10° and a further 35 days at 0° (●——●).

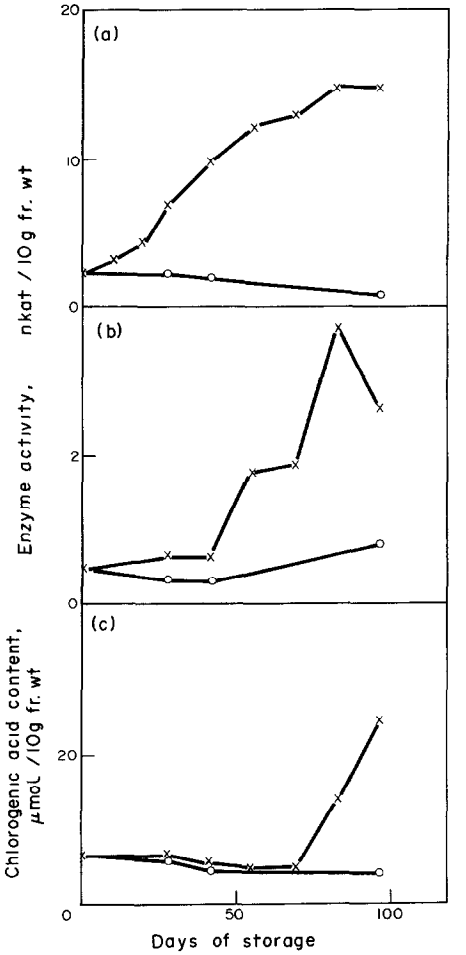

Fig. 4. Changes in the activities of CQT (a) and p-coumarate CoA ligase (b) and in the concentration of chlorogenic acid (c) in sweet potatoes stored for up to 98 days at either 7.5° (×——×) or 15° (O——O).

Table 2. Effect of storage atmosphere on the increase in CQT activity in potatoes stored at 0°

Storage period at 0° (days)	CQT activity (nkat/10 g fresh weight) Storage atmosphere							
	Air	N_2	1%O_2	3%O_2	5%O_2	5%CO_2	10%CO_2	15%CO_2
0	0·8	—	—	—	—	—	—	—
7	3.0	0.6	0.6	0.8	1.2	1.3	1.1	1.2
14	13.0	0.7	1.0	3.4	5.1	4.3	1.1	1.0

activity while at 7.5° there is a rapid rise reaching a maximal level 6.3 times the initial, after 90 days of storage. Similarly, *p*-coumarate CoA ligase [Fig. 4(b)] does not rise during storage at 15° while at 7.5° there is a rise to a peak after about 90 days following a lag phase of about 40 days. The levels of chlorogenic acid [Fig. 4(c)] rise during storage at 7.5° only after a long lag period while at 15° no increase in concentration during the 98 days period of storage occurred. No increases in PAL level were found in either storage condition. Lieberman *et al* [10] also showed that the level of chlorogenic acid rose in sweet potato tubers stored under temperature conditions inducing chilling injury but the lag phase before the level of chlorogenic acid rose was shorter than that observed in the present work. The kinetics of increase in CQT and *p*-coumarate CoA ligase are both somewhat different from the time course of increase in chlorogenic acid. Thus any relationship between the increased levels of enzymes and the accumulation of chlorogenic acid cannot be direct unless there is also a change of pattern of turnover of chlorogenic acid.

There are a number of situations in which potato tuber tissues can be stimulated to accumulate chlorogenic acid. The most studied of these is the ageing of tissue slices in the light at 20° which Zucker [11] showed greatly stimulated the accumulation of the product. Studies on the enzymes controlling this accumulation of chlorogenic acid showed that three enzymes, PAL [11], CA4H [12] and *p*-coumarate CoA ligase [13], were induced during ageing and the idea of a 'PAL operon' controlling the induction of a few controlling enzymes was proposed by Zucker [14]. Although this concept was thought by later workers [15] to be too simple a mechanism to explain differential effects on the induction of PAL and CA4H, it is clear that the major control of the biosynthesis of chlorogenic acid under these conditions is exerted by these three enzymes. The level of CQT does not change to any great extent during the ageing of disks of potato tuber in the light at 20° [16]. In the present work, in which treatment of whole tubers in the cold has been shown to induce chlorogenic acid accumulation, there are small increases in both PAL and *p*-coumarate CoA ligase which are of the order of 10 and 30% respectively of the increase found during 24 hour ageing in the light at 20° [see 16]. However, the increase in CQT occurring in whole tubers stored at 0° is more than 13 times that occurring in aged disks [see 16]. The increase in chlorogenic acid content of potato disks during ageing in the light for 24 hr is about 3 fold [17] and this is of the same order as the increase of 1.8–1.9 fold found during storage of whole tubers at 0°. The mechanisms of control of chlorogenic acid biosynthesis in the cold have yet to be elucidated but the initial findings suggest that these control mechanisms may be different from those controlling the synthesis of chlorogenic acid following mechanical damage in aged disks.

The data given in this paper taken together with our previously published work [1] show that, in three tissues, there is a low temperature induced rise in CQT activity and that the critical temperatures below which an increase is stimulated during storage of the tissue varies from commodity to commodity. The threshold temperature for increase in activity is between 11 and 13° for tomato (Rhodes and Wooltorton, unpublished data), between 7.5 and 15° for the sweet potato, and between 2 and 5° in the potato. The temperature characteristics required to induce an increase in CQT activity in the potato seem very similar to the temperature requirements for the induction of low temperature sweetening [6]. Both low temperature sweetening and the rise in CQT activity are inhibited by low O_2 atmospheres [present paper and 18]. It is interesting to speculate whether these two phenomena are not different expressions of the same basic changes occurring in the physiology of potato tubers at a critical temperature between 2 and 5°. CQT is not the only enzyme of the potato which has been shown to be induced in the cold. Pressey and Shaw [19] showed that invertase increased in potatoes stored at 4° and disappeared when such potatoes were returned to 18°. These authors correlated the changes in invertase activity with the appearance and disappearance of a proteinaceous invertase inhibitor [20] during the transfer between the two temperatures.

The significance of the rise in CQT in the three tissues studied in relation to low temperature metabolism is difficult to assess but in tomatoes and sweet potatoes it occurs at temperatures at which chilling injury [5] is also induced. In the potato, which is considered chilling insensitive, the rise in CQT and the accompanying rise in chlorogenic acid only occur at very low temperatures (about 2°). Rise in chlorogenic acid levels is often found in plants exposed to various types of stress [16] and here low temperature may exert a form of physiological stress. It seems likely that the rise in level of CQT may be a good indicator of metabolic changes under temperature stress conditions but the role the enzyme and its products play at these low temperatures requires further study.

EXPERIMENTAL

Potatoes (var. Majestic) were grown at Easton, Norfolk, harvested on 20 September 1976 and, after washing and grading, were stored at 10° for curing. For the various storage experiments, tubers were transferred to cabinets in rooms maintained at the required temp. A supply of fresh humidified air was continuously passed through each chamber. When atmospheres other than air were used, these were supplied from cylinders of the appropriate gas mixtures. At intervals, samples of 5–10 tubers were taken, peeled and the flesh tissue frozen in liquid N_2. The frozen tissue was ground to a homogenous powder at liquid N_2 temperature using an Ultraturrax homogeniser. Aliquots of this powder, stored in liquid N_2, were used for the extraction and assay of both the enzymes of phenylpropanoid metabolism and the level of chlorogenic acid. Sweet potatoes were obtained from a local supplier and were stored at 7.5 and 15° for up to 98 days. At intervals samples of tissue were taken and homogenized in liquid N_2 in the same way as for the potato. In the study of both potato and sweet potato, undamaged tubers were selected for analysis at each stage. At 0 the potatoes showed damage to some tubers with symptoms similar to those described by other workers [7, 8], developing after 2–3 months, but this did not increase in incidence with longer term storage. In the sweet potato no damage was observed except in the final sampling after 98 days at 7.5°. The methods for the purification of CQT on columns of DEAE-cellulose [3] and for the preparation of tissue extracts [1] are as previously published for the tomato. The assay methods used for *p*-coumarate CoA ligase [13], PAL [11] and CQT [3] are also as previously described.

Chlorogenic acid was extracted by three successive extractions of 10 g aliquots of the frozen tissue powder with a total vol. of 80 ml of 70% 2-propanol containing 1 mM EDTA and 5 ml HOAc/l. The extract was evapd *in vacuo* to remove the 2-propanol and the aq. phase extracted twice with 1 vol. Et_2O.

The ether phase after separation was washed with 1 vol. of acidified H_2O (1 ml 1N HOAc/l.) and the combined ether extracted aq. phase and washing were evapd *in vacuo* to 5 ml. The extract was made to 15 ml and 0.25, 0.5 and 1 ml aliquots were taken for the colorimetric estimation of chlorogenic acid by a method [21] which was modified to give a final reaction vol. of 3 ml.

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