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ISOENZYMES OF LIPOLYTIC ACYL HYDROLASE AND ESTERASE IN POTATO TUBER

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Key Word Index—*Solanum tuberosum*; Solanacae; potato; lipolytic acyl hydrolase; esterase; isoenzymes.

Abstract—Five varieties of potato (*Solanum tuberosum*) were shown by gel- and free-flow-electrophoresis to exhibit multiple forms of lipolytic acyl hydrolase (LAH) and esterase enzymes. The electrophoretic patterns of LAH and esterase activities and protein differed with the variety and were characteristic for a given variety. In the variety (Golden Wonder) with the highest LAH activity (*p*-nitrophenylpalmitate as substrate), this was 200-fold greater than the esterase activity (*p*-nitrophenylacetate as substrate) and isoenzyme patterns for both enzymes were the most complex. In the variety with a very low LAH activity (Désirée), the LAH and esterase activities were similar and more simple isoenzyme patterns for these enzymes were observed.

INTRODUCTION

MULTIPLE forms of esterase enzymes, catalysing the hydrolysis of carboxylic esters of short-chain acids, have been demonstrated in several plant tissues including leaves[1] and seeds[2-4] of *Phaseolus* species, needles and macrogametophytes of *Picea abies*,[5] seeds of pea[2,3,6] and cotton,[7] a range of citrus and curcubit fruits,[2] carrot roots,[8] legume root nodules[9] and tubers of *Solanum* species.[2,10-15] Characteristic electrophoretic patterns of soluble proteins and esterases have been demonstrated for tubers of individual varieties of potato (*Solanum tuberosum*).[10-15]

A carboxylic ester hydrolase (lipolytic acyl hydrolase, LAH) which acts on the endogenous phospholipids and galactolipids of the potato tuber has been isolated from this tissue[16-18] and shown to have properties similar to galactolipase [E.C. 3.1.1.26],

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lysophospholipase [E.C. 3.1.1.5] and 'phospholipase B'.[19] The partially purified enzyme hydrolysed the esters of short chain acids much less readily than long chain lipid esters.[17] Preliminary evidence[17] indicated that the esterase and lipolytic acyl hydrolase activities were not homogeneous.

The present work has shown that multiple forms of both esterase and lipolytic acyl hydrolase activities exist in potato tubers, that these activities are not due to the same enzyme forms and that the electrophoretic patterns of both enzymes differ with variety of potato.

RESULTS AND DISCUSSION

Enzyme levels

Five varieties of common commercial potatoes grown in the British Isles were studied. Previous work[18,20] had established that of these, four had high levels of LAH activity ranging from Golden Wonder (46–50 units (μmoles *p*-nitrophenylpalmitate hydrolysed/min)/g fr. wt) to Orion (5–9 units/g) and one, Désirée, had a relatively very low level of activity (0·06–0·20 units/g).

Esterase, LAH and acid phosphatase activities (relative to protein content of acetone powder preparations) were determined on three varieties. Table 1 shows that, whereas similar levels of acid phosphatase activity were found in each variety, differences were noted in the LAH and esterase activities of different varieties. The esterase activity of the Désirée variety was approximately half that of the high-LAH varieties whereas the LAH activity of Désirée was only about 0·3% that of the high activity varieties. The LAH activity of Golden Wonder was some 200× greater than the esterase activity of this variety whereas the Désirée variety had comparable levels of these two enzyme activities.

TABLE 1. COMPARISON OF LAH, ESTERASE AND ACID PHOSPHATASE ACTIVITIES IN TUBERS OF DIFFERENT POTATO VARIETIES

Potato variety	Enzyme: substrate:	Enzyme specific activity LAH *p*-nitrophenylpalmitate (units)	Esterase *p*-nitrophenylacetate (units × 10^2)	Acid phosphatase *p*-nitrophenylphosphate (units × 10^2)
Golden Wonder		4·3	1·9	7·2
Pentland Crown		3·3	1·9	9·8
Désirée		0·012	0·96	10·5

For assay details see Experimental. Several concentrations of each enzyme preparation (15,000 *g* supernatant) established a linear activity [enzyme] response. Enzyme units refer to μmoles of substrate hydrolysed/min per mg of protein.

Free-flow electrophoresis patterns

Solutions containing acetone powder preparations from each of the five varieties together with added marker dyes were subjected to free-flow electrophoresis at pH 8·3. Figure 1 shows the patterns obtained for protein (E_{280}), LAH and esterase (2-naphthylacetate hydrolase) activities.

The three varieties with highest LAH activity (Golden Wonder, Pentland Crown and Majestic) all show LAH and esterase peaks close to, but not quite coincident with, a major

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protein peak. In these varieties a close coincidence of the LAH and esterase peaks was observed. The Orion variety (moderate LAH activity) showed less coincidence of the LAH and esterase peaks and greater separation of these from the major protein peak. The very low LAH-activity variety. Désirée, had a quite different electrophoretic profile; two peaks of enzyme activity were obtained; one (fractions 29–32) having a much higher ratio of LAH to esterase activity than the second (fractions 22–28). However, a peak of LAH activity was coincident with the "esterase" peak (fractions 22–28) and a peak of esterase activity was coincident with the LAH peak (fractions 29–32). As with the other varieties, the predominant peak of LAH activity in Désirée occurred close to the major protein peak. However, the "esterase" peak was clearly separated from the main protein and LAH peaks.

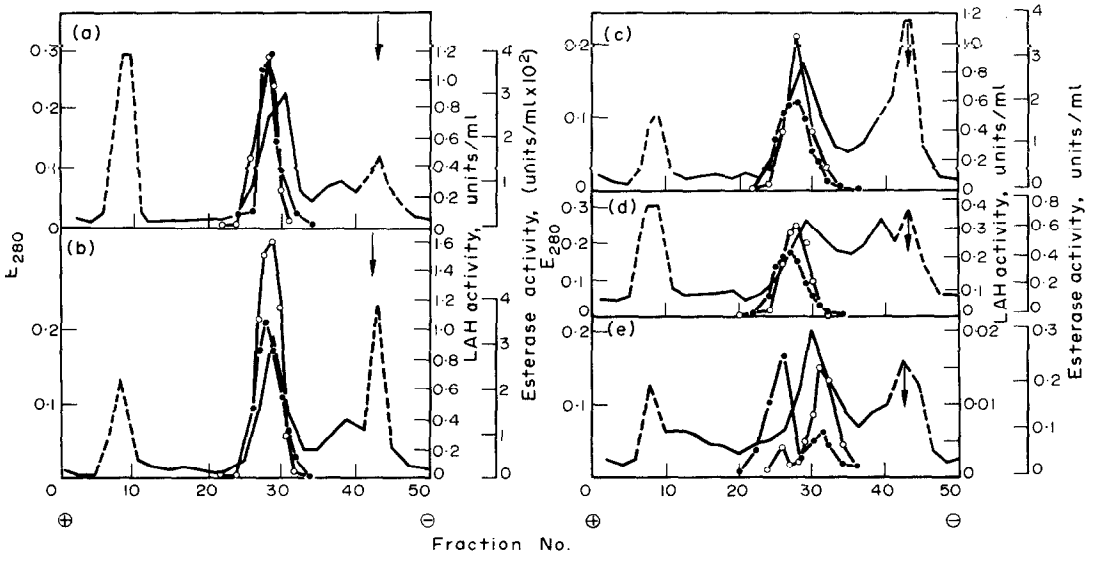

FIG. 1. FREE FLOW ELECTROPHORESIS PROFILES FOR LAH ESTERASE ACTIVITIES OF DIFFERENT VARIETIES OF POTATO.

Acetone powder preparations (5–8 mg of protein) in 1–1·8 ml of 0·2 M Tris-acetate buffer, pH 8·3, were injected at a rate of 1 ml/hr at entry point 7 (represented by arrow in Fig. 1) of the electrophoresis equipment. DNP-aspartate and DNP-ethanolamine were used as marker compounds. LAH activity (O—O) of fractions was determined on aliquots (30–200 μl) by incubation for 10–30 min. (time and enzyme concentration depending on activities of individual varieties) with *p*-nitrophenylpalmitate (see Experimental). Similarly 200–800 μl aliquots were assayed (45–60 min) for esterase (2-naphthylacetate hydrolase) activity (●——●). The continuous line represents E_{280} readings. (Broken regions represent absorptions due to DNP-aspartate and DNP-ethanolamine marker compounds). Enzyme units refer to μmoles substrate hydrolysed/min. The varieties used were: (a) Golden Wonder, (b) Pentland Crown, (c) Majestic, (d) Orion and (e) Désirée.

Acrylamide gel electrophoresis

The above results of the free-flow electrophoresis studies indicated non-homogeneity of LAH and esterase enzymes, at least for the varieties with lower levels of these enzymes. Disc gel electrophoretic patterns for protein, together with LAH and esterase activities, were determined on acetone powder preparations of tubers from the five varieties of potato.

Standard staining methods were used to detect protein and esterase bands on acrylamide gels; however, the published method of staining for "lipase" activity (using α-naphthyl

nonanoate as substrate)[21] did not work in our hands for detecting LAH bands. Thus protein and esterase were detected on intact gels and LAH and esterase activities were determined after slicing the gels and measuring each enzyme in the halved slices.

Figure 2 shows a diagram of protein and esterase profiles obtained by staining gels run concurrently on preparations from each variety. The profiles of both protein and esterase were reproducible for a given variety and compared well with profiles obtained on three of the same varieties but grown in the following year at a different location and used for other studies.[22] These observations were consistent with published work on electrophoretic profiles of protein and esterase in tubers of other European[10,12] and North American[11,13–15] potato varieties. Figure 2 illustrates the multiple (5) esterase bands in the two high LAH varieties (Golden Wonder and Pentland Crown), 3 esterase bands in Orion (moderate LAH activity) and a single band only for the low-LAH, Désirée variety. Comparison of protein and esterase patterns showed corresponding protein-staining bands for all esterase bands; a similar observation was made for a wide range of wild types and cultivated varieties of tuber bearing *Solanum* species.[13]

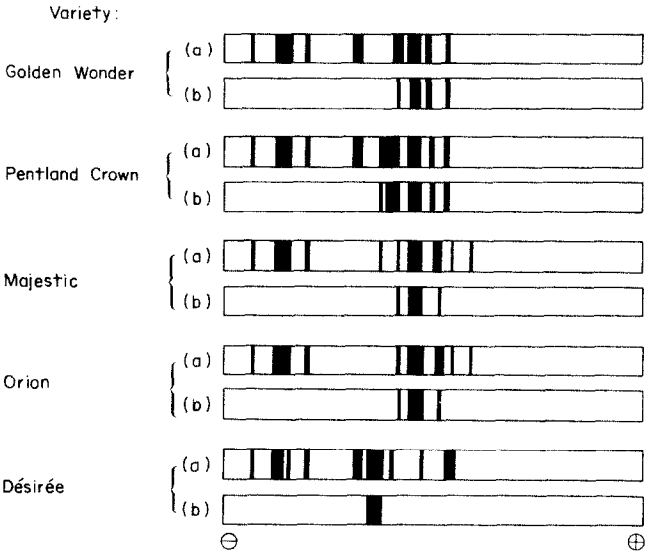

FIG. 2. POLYACRYLAMIDE DISC GEL ELECTROPHORESIS TO SHOW PROTEIN AND ESTERASE PATTERNS IN TUBERS OF DIFFERENT VARIETIES OF POTATO.

The diagram represent tracings of gels stained for (a) protein or (b) esterase activity of acetone preparations of each variety. All the gels were run simultaneously at pH 8·3 as described in Experimental. Approx. 0·2 mg protein was applied to each gel.

Enzyme determinations on approx. 1-mm slices obtained from a portion of acrylamide gels are given in Fig. 3. The LAH profile (open circles) shows that several electrophoretic forms of this enzyme exist in the four varieties with high or moderate LAH activity. As in the case of the esterase enzyme, the Désirée variety showed a more simple LAH profile with one major peak. Again, the LAH profiles were reasonably reproducible for given varieties grown in different years and at different locations.[22] The esterase activity in halved 1-mm gel slices was low and the esterase profiles (closed circles in Fig. 3) were not sufficiently

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reproducible to permit close comparisons between peaks of LAH and esterase activities; nevertheless, the general esterase pattern supports the results obtained by staining techniques and by free-flow electrophoresis.

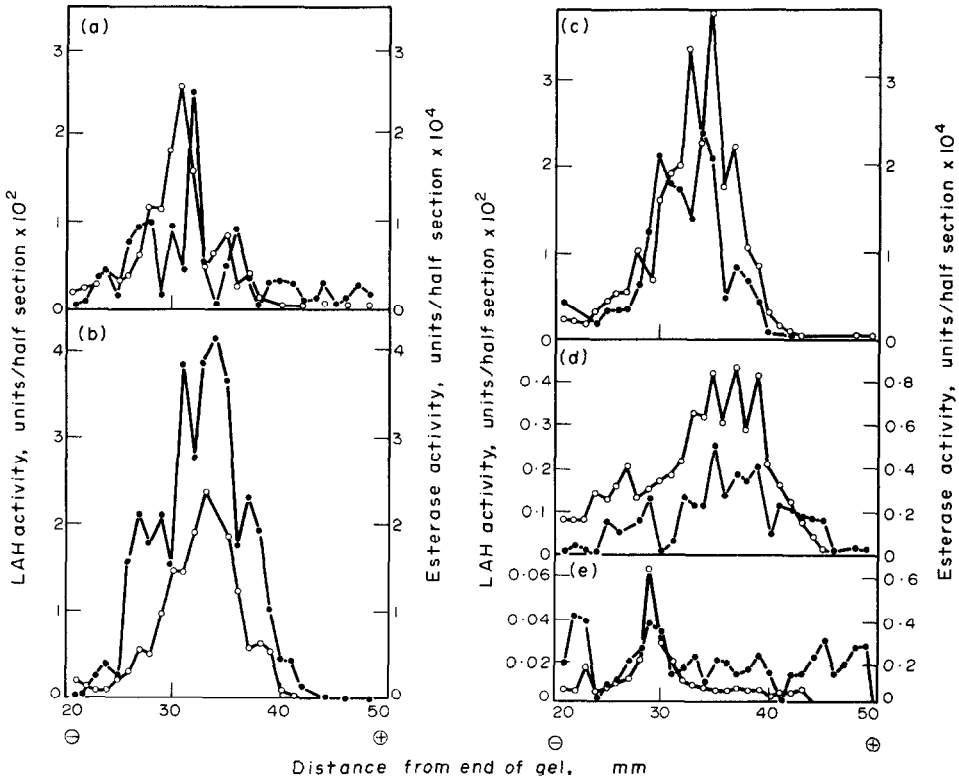

FIG. 3. ESTERASE AND LAH ACTIVITY DISTRIBUTION FOLLOWING ELECTROPHORESIS ON POLYACRYLAMIDE GELS.

The portion of gels between 2 and 5 cm from the cathode end was cut into approx. 1 mm slices which were then halved and assayed for enzyme activity on *p*-nitrophenylpalmitate (LAH; O—O) or 2-naphthylacetate (esterase; ●—●). Incubation periods (see Experimental for details) were determined by the enzyme activity of each variety and for LAH ranged from 15 min (Golden Wonder) to 105 min (Désirée). Approx. 3–4 hr incubations were necessary for esterase measurements. Enzyme units refer to μmoles substrate hydrolysed/min. The varieties used were (a) Golden Wonder, (b) Pentland Crown, (c) Majestic, (d) Orion and (e) Désirée.

Conclusions

The present work has confirmed, for different varieties of potato, previous observations on protein and multiple esterase electrophoretic profiles.[10–15] In addition, the enzyme, lipolytic acyl hydrolase, which, in some varieties, has an activity some 200× greater (*p*-nitrophenylpalmitate as substrate) than the esterase (*p*-nitrophenylacetate as substrate; Table 1), also exists in multiple forms which show varietal differences. Comparisons of electrophoretic patterns (Figs. 1–3) do not give evidence for absolute specificities of separate esterase and LAH enzymes but indicate that the different forms of esterase and LAH have differing affinities for short- or long-chain esters.

Desborough and Peloquin[13] have postulated that the *Solanum* esterase isoenzymes are different forms of a tetramer composed of 1 to 3 types of monomer and that varietal

phenotypes are genetically determined. Although studies on many varieties and hybrids would be necessary to show an analogous case for the LAH enzyme, the size (molecular weight approx. 10^5)[17] and electrophoretic properties of the enzyme described above do not preclude a similar phenomenon. The work described here was performed with mature tubers stored for a limited period of time. However, the LAH activity of a given variety remains relatively constant throughout the life-cycle of the tuber.[20]

EXPERIMENTAL

Materials. Potatoes (*Solanum tuberosum*) of the varieties Désirée, Golden Wonder, Majestic, Orion and Pentland Crown were grown locally under standard agricultural conditions and harvested in September 1971. Tubers were stored dark at 6°.

Enzyme extracts. Tuber homogenates were prepared as previously[18] in 2 vol. of water containing 2 mM $Na_2S_2O_5$ and the supernatant obtained by centrifugation at 15 000 *g* for 30 min was retained. Acetone powders were prepared as previously[17] from the 15 000 *g* supernatant preparations.

Enzyme assays. Standard methods as previously used[17] were employed for esterase (2-naphthylacetate and *p*-nitrophenylacetate hydrolases) and acid phosphatase determinations. Lipolytic acyl hydrolase (LAH) activity was determined, using *p*-nitrophenylpalmitate as substrate either by a continuous recording spectrophotometric method[17] (for enzyme preparations) or by incubation for measured times and subsequent determination of the *p*-nitrophenol released[17] (for fractions obtained in electrophoretic separations).

Acrylamide gel electrophoresis. Gel preparation sample loading, electrophoresis and staining for protein and esterase (2-naphthylacetate hydrolase) activity were described previously.[17] A McIlwain tissue slicer (Mickle Laboratories Engineering Co., Gomshall, Guildford, Surrey) was adjusted to out 1 mm slices of the portion of the gels between 2 and 5 cm from the cathode end. The slices were bisected and each half placed in 0·5 ml H_2O at 0° and left for 1–16 hr to extract enzyme from the gels. Then *p*-nitrophenylpalmitate or 2-naphthylacetate, together with appropriate buffers for LAH and esterase assays respectively, were added and enzyme activities determined as above.

Free-flow electrophoresis. The apparatus and method for continuous free-flow electrophoresis were described previously.[17]

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