Characterization of the lipid acyl hydrolase activity of the major potato (*Solanum tuberosum*) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector

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Patatin is a family of glycoproteins that accounts for 30–40 % of the total soluble protein in potato (*Solanum tuberosum*) tubers. This protein has been reported not only to serve as a storage protein, but also to exhibit enzymic activity. By using a baculovirus system to express protein from the patatin cDNA clone pGM01, it was unambiguously shown that the patatin coded by this DNA has lipid acyl hydrolase and acyltransferase activities. The enzyme is active with phospholipids, monoacylglycerols and *p*-nitrophenyl esters, moderately active with galactolipids, but is apparently inactive with di- and tri-acylglycerols.

INTRODUCTION

Patatin is the trivial name given to a family of glycoproteins that makes up over 30 % of the total soluble protein in potato (*Solanum tuberosum*) tubers. Patatin has been found in all potato cultivars thus far examined, including the South American genotypes andigena and phureja. The isoforms of patatin have an apparent M_r of approx. 40 000 as judged by SDS/PAGE, but they can be separated on non-denaturing gels or by isoelectric focusing. Most potato cultivars contain 12–15 patatin species that are immunologically identical both within and between cultivars [1]. The similarity of patatin isoforms has been shown by amino-acid-sequence analysis and by homology of the various patatin cDNAs [1,2].

Patatin accumulates in large amounts in tubers, but is not normally found in other plant organs. However, under certain conditions it can be induced to accumulate to high levels in stems and petioles [3]. This accumulation occurs in the absence of the normal morphogenesis and cell proliferation that accompany tuber formation and under conditions that are non-inductive for tuber formation, e.g., long-day photoperiod. Accumulation of patatin in tubers, stems and petioles is blocked by low concentrations of gibberellic acid [4].

In addition to the major isoforms of patatin, there is also an immunologically distinct form of patatin present in small amounts in roots. These two classes of patatin correspond to two different classes of genes which are distinguished by the presence (Class II) or absence (Class I) of a 22-bp insertion in their 5' untranslated regions [5]. Class-I patatin genes encode the major patatin isoforms in tubers, whereas Class-II genes encode the root form of patatin.

Patatin appears to serve as a storage protein, but also has been reported to have esterase activity with a large number of lipid substrates [6]. This finding was confirmed by Racusen [7] and Galliard [8], who proposed that patatin was a lipid acyl hydrolase. Hasson & Laties [9] resolved three distinct lipid-degrading enzyme activities

and reported a 25-fold purification of the proteins, whereas Shepard & Pitt [10] reported that the lipid acyl hydrolase activity was due to two enzyme species. Hirayama *et al.* [11] reported a 350-fold increase in lipid acyl hydrolase activity from potato tubers, but, as pointed out by Racusen [7], the final specific activities reported by these investigators were no higher than that reported by Galliard [8].

Although purification of patatin is straightforward, assignment of its lipid acyl hydrolase activity is not a trivial task, since other esterases might co-purify with patatin. To determine unambiguously whether patatin has lipid acyl hydrolase activity, the helper-independent baculovirus expression vector *Autographa californica* nuclear polyhedrosis virus (AcNPV) [12] has been employed to express the patatin cDNA clone pGM01 [2]. This has allowed us to characterize the enzymic activity of a single isoform. Production of a protein in a foreign cellular environment also allows discrimination between activities which are due to this isoform and those which are due to other co-purifying factors. We use this system to produce and secrete large quantities of a biologically active plant enzyme from insect cells. This work unequivocally shows that an isoform of patatin corresponding to the cDNA clone pGM01 displays lipid acyl hydrolase and acyltransferase activities.

EXPERIMENTAL

Patatin purification, immunological detection and quantification

Patatin_{tuber} was extracted and purified from *Solanum tuberosum* L. (cv. Superior) by the method of Racusen & Foote [13], except that 0.1 g of polyvinylpyrrolidone/g of tubers was added before homogenization. Protein was estimated by the dye-binding method of Bradford [14], with bovine serum albumin (fraction V, Sigma) as the standard, and patatin was quantified by rocket immunoelectrophoresis [15].

SDS/PAGE was performed as described previously

Abbreviations used: PAGE, polyacrylamide-gel electrophoresis; AcNPV, *Autographa californica* nuclear polyhedrosis virus; PBS, phosphate-buffered saline (0.01 M-sodium phosphate/0.14 M-NaCl, pH 7.1).

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[16]. Separated proteins in the gel were transferred to nitrocellulose and immunologically stained [17] by treatment first with patatin antibody, followed by goat anti-rabbit antibody conjugated to horseradish peroxidase (Bio-Rad) [1,5].

Recombinant DNA cloning procedures

Standard recombinant DNA techniques [18] were used for the production of recombinant plasmids. Restriction enzymes were obtained from Bethesda Research Laboratories. The protein-coding region plus 24 bp of the flanking 5' untranslated region was removed intact from the patatin cDNA clone pGM01 by enzymic digestion with restriction endonuclease *Dra*I. This fragment was cloned into the *Sma*I site of pUC18, subsequently removed as an *Eco*R1/*Pst*I fragment, and then cloned into the transfer vector pAc610. This transfer vector has a multiple cloning site from M13mp10 [19] cloned into the *Sma*I site of pAc461 [20] as well as a deletion of sequences around the unique *Eco*R1 site of pAc461 to yield a transfer plasmid of about 8.2 kb. The resultant plasmid, pAc610-GM01, has polyhedrin sequences to -7 bases before the translation start site, followed by 26 bases of the polylinker and the patatin cDNA. The patatin-coding region is followed by the remainder of the polyhedrin gene, beginning at position +670, with its accompanying poly(A) addition sites.

Recombinant patatin production and expression from virus-infected insect cells

Spodoptera frugiperda (fall armyworm) [21] Sf9 cells were grown as spinner or monolayer cultures in Hink medium [22] supplemented with 10% (v/v) fetal-bovine serum (Hazleton) at 28 °C. Recombinant virus was produced in the Sf9 cells by co-transfection with wild-type viral DNA and pAc610-GM01. After 3 days incubation, the supernatant fraction from these cells were assayed for plaque production on fresh cultures of Sf9 cells overlaid with medium containing 1.5% (w/v) low-melting-temperature agarose. After 5–6 days, virus-infected cells were selected for occlusion-minus (*occ*-) plaques. In all, 13 presumptive *occ*- plaques were picked and transferred to individual wells of a 96-well plate. Each well was supplied with 1.5×10^4 Sf9 cells in 100 μ l of medium. The cells were incubated under standard conditions for 2 days. After removal of the medium, cells in each well were lysed in 200 μ l of 0.5 M-NaOH and the lysed cell suspensions were then neutralized. The lysed cell suspensions were transferred to nitrocellulose, and immobilized DNA was hybridized with the nick-translated (32 P-labelled) patatin cDNA clone [23]. Viral stocks were produced from six recombinant viruses which gave the strongest hybridization signals when exposed to X-ray film. One recombinant viral stock was used to infect insect cells in individual wells of a 6-well plate. Each well was supplied with 1.5×10^6 cells in 1.6 ml of standard medium. After 48 h incubation, the medium was removed, combined, and stored at -20 °C. The cells were harvested, combined and rinsed with PBS, and finally resuspended in PBS at a concentration of 4.0×10^6 cells/ml and assayed immediately for enzymic activities as described below. The expressed protein corresponding to the cDNA GM01 contained in the medium from the virus-infected cells is designated as 'patatin_{GM01}'.

Esterase assay

Esterase activity was determined by a modification of the procedure described previously [7]. All substrates were obtained from either Sigma or Supelco. A unit of activity is defined as that releasing 1 μ mol of fatty acid/min. The A_{400} was measured at 10 min intervals, beginning with the addition of the substrate solution. Assay mixtures contained 2.5 ml of 0.1 M-Tris/HCl, pH 8.2, 0.05 ml of enzyme solution (either patatin_{GM01} or patatin_{tuber}) and 1.0 ml of substrate solution. All substrate solutions contained 87 μ mol of substrate in 100 ml of 1.0% (v/v) Triton X-100 + 1.7 ml 1% (w/v) SDS. Carbon-chain-length assays included 50 μ l of medium containing patatin_{GM01}, 50 μ l of medium from wild-type infected cells, 50 μ l of medium from uninfected cells, or 1 μ g of purified patatin_{tuber} in 50 μ l of 0.1 M-Tris/HCl, pH 8.2.

Lipid acyl hydrolase assay

Lipid acyl hydrolase was assayed as described by Hirayama *et al.* [11]. Assays were performed in a 1 ml volume containing 0.1 M-buffer (either citrate, pH 5.0, or Tris/HCl, pH 8.2), enzyme and 0.1 ml of substrate stock solution, which was sonicated to form a uniform dispersion. Substrate stock solutions (10 ml) contained 50 μ mol of hydrolysable bond equivalents in 0.1 mmol of sodium deoxycholate or 1 or 4.0% (v/v) Triton X-100. Mixtures contained 1 μ g of either patatin_{tuber} or patatin_{GM01}. Mixtures containing patatin_{tuber} were supplemented or not with 128 μ l of medium derived from wild-type infected cells, which is the volume equivalent to 1 μ g of patatin_{GM01} in the medium derived from the recombinant-virus-infected cells. Incubation times (at 27 °C) were 10 min for mono-olein (mono-oleoylglycerol), 30 min for phosphatidylcholine, monopalmitin (monopalmitoylglycerol), and monogalactosyldiacylglycerol, and 120 min for di- and tri-acylglycerols. Reactions were stopped by adding 5 ml of a solvent mixture of *n*-hexane/ethanol/1.5 M-H₂SO₄ (400:200:1, by vol.) and the mixture was shaken and centrifuged. Non-esterified fatty acids in the hexane layer were determined by the rhodamine method described previously [24].

Chromatography

Fatty acids for the thin-layer-chromatographic analysis were isolated as described for the routine lipid acyl hydrolase assay, except that rhodamine was omitted [24]. The hexane layer was collected, evaporated to a small volume and chromatographed on activated silica-G plates and developed with *n*-hexane/diethyl ether (9:1, v/v). Plates were sprayed with concentrated H₂SO₄ and heated briefly at 152 °C. Charred material resolved on the chromatogram was identified to lipid class by comparison with the migration distance of lipid standards.

RESULTS

Patatin expression from the baculovirus vector

In order to produce and express patatin from the viral-infected insect cells, the recombinant plasmid pAc610-GM01 (cloning strategy outlined in Scheme 1) and wild-type viral DNA were co-transfected into the Sf9 cultured cells. *occ*- plaques from lawns of infected cells were picked, DNA from the viral particles was extracted, and

Scheme 1. Construction of the patatin transfer vector pAc610-GM01

To construct the patatin viral expression vector, Ac610-GM01, the patatin coding sequence was removed from the cDNA clone pGM01 by digestion with *Dra*I. This fragment, which contains 24 bp of 5' untranslated sequence, was then cloned into the *Sma*I site of pUC 18, removed as an *Eco*R1/*Pst*I fragment and cloned into the transfer vector pAc610. The resulting plasmid, pAc610-GM01, has polyhedrin sequences to -7 bases before the translation start site, followed by 26 bases of polylinker and the patatin cDNA. The patatin cDNA sequence is followed in tandem by the remainder of the polyhedrin gene beginning at +670 with its accompanying poly(A) addition sites.

Fig. 1. Immunoblot of recombinant patatin

Lane 1, medium from Ac610-GM01-infected cells ($7.8 \mu\text{g}$ of patatin/ml); lane 2, lysate from wild-type-infected cells (equivalent to 4.0×10^6 cells/ml); lane 3, lysate from wild-type-infected cells plus $0.5 \mu\text{g}$ of patatin_{tuber}; lane 4, lysate from Ac610-GM01-infected cells (4.0×10^6 cells/ml).

the nucleic-acid-containing solution was transferred to nitrocellulose paper and incubated with a specific probe for patatin, the cDNA GM01 [2]. DNA from six patatin-producing viral clones gave strong hybridization signals, of which one clone (H-1) was chosen for further study. The amount of patatin produced from a standard quantity of cultured cells as outlined in the Experimental section and determined by rocket gel immunoelectrophoresis was $7.8 \mu\text{g}/\text{ml}$ of medium, whereas patatin retained in the infected cells was $0.6 \mu\text{g}/\text{ml}$. Therefore approx. 95% of the expressed patatin is excreted by the cells into the culture medium.

Patatin_{GM01} retained in the viral-infected insect cells and expressed into the culture medium was compared with patatin_{tuber} by SDS/PAGE and immunoblotting with antibody specific for patatin (Fig. 1). The apparent M_r of patatin_{GM01} from both the infected cells and medium matched that of patatin_{tuber}. However, as expected, the patatin_{tuber} was more heterogeneous [1]. No patatin-specific bands were detected in extracts from wild-type viral-infected cells or their medium. Some lower- M_r species were detected in the patatin_{GM01} isolated from infected cells, whereas the enzyme secreted into the medium was homogeneous (Fig. 1). It is not known whether the lower- M_r species isolated from the insect

Fig. 2. Influence of fatty acid chain length on hydrolysis of *p*-nitrophenyl fatty acid esters

Esters ranging in carbon chain lengths from 2 to 18 were incubated with medium from cells infected with the recombinant virus Ac610-GM01 (●), patatin_{tuber} (○), medium from wild-type-virus-infected cells (■) and medium from uninfected cells (□). Values for activity are the means of three determinations.

cells represent unprocessed forms of the secreted protein or are degradation products. For this reason, we chose to continue the characterization of the enzymic activity of patatin_{GM01} using the protein secreted by the Ac610-GM01-infected cells.

Carbon-chain-length specificity

The enzymic activity of patatin_{GM01} was first compared with that of patatin_{tuber} in esterase assays with various *p*-nitrophenyl fatty acid esters having carbon chain lengths which ranged from 2 to 18. Medium from uninfected insect cells and from cells infected with wild-type virus contained small, but measurable, amounts of short-chain esterase activity (Fig. 2). This activity diminished with increasing carbon chain length and disappeared with acyl esters having carbon chain lengths greater than 10. In contrast, the medium from cells infected with Ac610-GM01 (patatin_{GM01}) displayed a biphasic pattern of activity, with highest activities in mixtures containing 10- and 16-carbon-chain-length acyl esters. This specificity paralleled that obtained with patatin_{tuber}. Although the carbon-chain-length specificities of patatin_{GM01} and patatin_{tuber} are the same, the specific activity of patatin_{GM01} on both 10- and 16-carbon acyl esters was found to be approximately twice that of patatin_{tuber} (results not shown).

Specific activity with other lipid substrates

Having demonstrated that patatin_{GM01} and patatin_{tuber} exhibit an optimal esterase activity with two narrow ranges of *p*-nitrophenyl fatty acid esters, we compared the specific activities of these two patatin sources on various phospholipids, a galactolipid, and on mono-, di- and tri-

Table 1. Cleavage of lipids by patatin expressed from Ac610-GM01 and by purified patatin isolated from tubers

Various classes of lipids were incubated with medium from Ac610-GM01-infected cells (Patatin_{GM01}), patatin purified from cv. Superior tuber tissue, and patatin purified from cv. Superior supplemented with medium from uninfected cells. The reaction mixtures contained either 1.0 μ M-sodium deoxycholate or Triton X-100 (0.1% or 0.4%, v/v).

Detergent	Patatin source	Lipid...	Lipid-class-hydrolysing activity (units/mg of patatin protein) [mean $\pm$ S.E.M. (n)]					
			Phosphatidylcholine				Monogalactosyl-diacylglycerol	
			Monopalmitin	Mono-olein	Dipalmitoyl	2-Oleoyl-3-palmitoyl	2-Palmitoyl-3-oleoyl	
0.1 μ M-Deoxycholate	Patatin _{GM01}		2.36 $\pm$ 0.25 (5)	45.70 $\pm$ 0.30 (2)	13.51 $\pm$ 0.91 (6)	3.84 $\pm$ 0.16 (2)	2.14 $\pm$ 0.04 (2)	0.04 $\pm$ 0.01 (2)
	Patatin _{tuber}		0.46 $\pm$ 0.07 (3)	28.85 $\pm$ 0.15 (2)	2.07 $\pm$ 0.14 (7)	12.71 $\pm$ 0.05 (2)	4.69 $\pm$ 0.01 (2)	0.26 $\pm$ 0.01 (2)
	Patatin _{tuber} + medium		1.25 $\pm$ 0.07 (2)	48.90 $\pm$ 0.10 (2)	12.31 $\pm$ 0.29 (5)	12.90 $\pm$ 0.15 (2)	6.24 $\pm$ 0.06 (2)	0.04 $\pm$ 0.01 (2)
0.1% Triton X-100	Patatin _{GM01}		4.59 $\pm$ 0.15 (2)	39.20 $\pm$ 0.02 (2)	9.18 $\pm$ 0.12 (5)	1.71 $\pm$ 0.0 (2)	—	0.23 $\pm$ 0.0 (2)
	Patatin _{tuber}		1.07 $\pm$ 0.15 (3)	26.41 $\pm$ 0.31 (2)	2.69 $\pm$ 0.02 (2)	4.72 $\pm$ 0.02 (2)	—	0.10 $\pm$ 0.02 (2)
	Patatin _{tuber} + medium		3.50 $\pm$ 0.24 (2)	42.00 $\pm$ 0.0 (2)	5.41 $\pm$ 0.11 (5)	5.24 $\pm$ 0.06 (2)	—	0.33 $\pm$ 0.0 (2)
0.4% Triton X-100	Patatin _{GM01}		5.17 $\pm$ 0.16 (2)	—	1.20 $\pm$ 0.06 (2)	—	—	—
	Patatin _{tuber}		1.48 $\pm$ 0.32 (2)	—	1.79 $\pm$ 0.04 (2)	—	—	—
	Patatin _{tuber} + medium		3.54 $\pm$ 0.13 (2)	—	1.72 $\pm$ 0.01 (2)	—	—	—

Table 2. Effect of pH on lipid acyl hydrolase activity

Monogalactosyldiacylglycerol and dipalmitoyl phosphatidylcholine were incubated with medium from Ac610-GM01-infected cells (patatin_{GM01}), patatin purified from cv. Superior tuber tissue and patatin purified from cv. Superior supplemented with medium from uninfected insect cells. The reaction mixtures contained either 0.1 μ M-sodium deoxycholate or 1.0% (v/v) Triton X-100.

Detergent	Patatin source	Lipid... pH...	Activity (units/mg of patatin protein) [mean $\pm$ S.E.M. (n)]			
			Monogalactosyldiacylglycerol		Dipalmitoyl phosphatidylcholine	
			5.0	8.2	5.0	8.2
0.1 μ M-Deoxycholate	Patatin _{GM01}		0.40 $\pm$ 0.0 (2)	0.04 $\pm$ 0.01 (2)	1.10 $\pm$ 0.02 (2)	13.5 $\pm$ 0.91 (6)
	Patatin _{tuber}		0.05 $\pm$ 0.01 (2)	0.26 $\pm$ 0.01 (2)	1.10 $\pm$ 0.02 (2)	2.07 $\pm$ 0.14 (7)
	Patatin _{tuber} + medium		0.42 $\pm$ 0.01 (2)	0.04 $\pm$ 0.01 (2)	4.70 $\pm$ 0.05 (2)	12.31 $\pm$ 0.29 (5)
0.1% Triton X-100	Patatin _{GM01}		0.18 $\pm$ 0.01 (2)	0.23 $\pm$ 0.0 (2)	3.30 $\pm$ 0.02 (2)	9.18 $\pm$ 0.12 (5)
	Patatin _{tuber}		0.01 $\pm$ 0.0 (2)	0.10 $\pm$ 0.02 (2)	2.80 $\pm$ 0.08 (2)	2.69 $\pm$ 0.02 (2)
	Patatin _{tuber} + medium		0.35 $\pm$ 0.05 (2)	0.33 $\pm$ 0.0 (2)	3.70 $\pm$ 0.02 (2)	5.41 $\pm$ 0.11 (5)

acylglycerols. Table 1 shows remarkable differences in the specific activity of patatin_{tuber} against the different classes of lipid substrates, with mono-olein serving as the most potent substrate. The di- and tri-acylglycerol compounds all gave less than 0.02 unit/mg of patatin, which is, however, above the detectable limit of this assay (0.01 unit/mg of patatin). In contrast with a published report [8], the detergent environment in the mixture minimally affected the enzyme activity, except in the case where 2-oleoyl-3-palmitoyl phosphatidylcholine was tested. Most strikingly, when medium (equivalent volume to patatin_{GM01}) recovered from the wild-type-infected insect cells was added to mixtures containing patatin_{tuber}, generally an enhanced lipid cleavage was seen with all the lipid substrates used. Therefore no potential inhibitory substances from medium derived from the cultured cells appeared to be present. The only instance where the activity of patatin_{GM01} differed significantly from that of patatin_{tuber} was in the presence of the phosphatidylcholine-type lipids. With dipalmitoyl phosphatidylcholine, the cleavage activities of both patatin sources in the presence of identical amounts of insect-cell medium were the same. Substitutions at either the 2 or 3 position of this lipid substrate reduced activity of the recombinant-produced patatin approx. 3-fold over patatin_{tuber}.

Table 2 shows the effect of pH on the hydrolysis of phosphatidylcholine and monogalactosyldiacylglycerol. Phosphatidylcholine was hydrolysed more rapidly by both patatin_{GM01} and patatin_{tuber} at basic pH than under acidic conditions in the presence of either Triton X-100 or sodium deoxycholate. In contrast, both patatin_{GM01} and patatin_{tuber} were more active on monogalactosyldiacylglycerol at low pH in the presence of sodium deoxycholate. The native and recombinant enzymes also gave similar pH-response curves in esterase assays (results not shown).

The lipid acyl hydrolase activities of patatin_{GM01} and patatin_{tuber} were further examined by comparing the effect of dipalmitoyl phosphatidylcholine concentration on reaction velocities. As shown in Fig. 3, neither patatin_{GM01} nor patatin_{tuber} had significant activity when

Fig. 3. Effect of dipalmitoyl phosphatidylcholine concentration ([S]) on lipid acyl hydrolase activity (v)

Assay mixtures contained either 1 μ g of patatin_{GM01} (●) or 1 μ g of patatin_{tuber} (○). Incubation conditions and lipid acyl hydrolase assays were as described in the Experimental section. Values for activity are the means of three determinations.

the concentration of this phosphatidylcholine was below 0.25 mM, but the activity of both increased rapidly as the substrate concentration increased to 1.5 mM. When the data were analysed in terms of the Hill equation, we calculated an apparent Hill coefficient (h_{app}) of 2.5 for both, with a co-operativity index of 4.0. Both enzymes also were inhibited by high substrate concentrations to

Fig. 4. T.L.C. of acyltransferase reaction products

2-Oleoyl-3-palmitoyl phosphatidylcholine was cleaved with 1 μ g of recombinant patatin without methanol (lane 1) and with 10% (v/v) methanol (lane 2). This substrate was also cleaved with 1 μ g of patatin_{tuber} supplemented with medium from wild-type-infected cells without methanol (lane 3) and with 10% (v/v) methanol (lane 4). Lipid standards (Std) include sterols (S), methyl esters (M), triacylglycerols (T) and fatty acids (F). Mixtures containing patatin_{tuber} were incubated for 30 min, and those containing patatin_{GM01} were incubated for 120 min.

virtually the same extent. In general, the behaviour of recombinant patatin and native patatin appear the same.

Acyltransferase activity

Galliard [25] reported that the potato lipid acyl hydrolase also possessed an acyltransferase activity. Addition of methanol to the lipid acyl hydrolase reaction mixture permitted the formation of methyl esters with 30% (v/v) methanol, leading to a maximum of 75% methyl ester conversion of the original substrate. We investigated whether the hydrolysed products of 2-oleoyl-3-palmitoyl phosphatidylcholine could be methylated. We found that both patatin_{GM01} and patatin_{tuber} catalysed the formation of methyl ester compounds that co-migrated with authentic methyl ester standards on thin-layer chromatograms (Fig. 4). No methylated products were detected by these methods when methanol was omitted from the mixture. Methyl ester formation was 4-fold less with patatin_{GM01} as compared with patatin_{tuber}. This approximates to the acyl-hydrolase-activity difference seen with these two patatin sources in reaction mixtures containing this phosphatidylcholine. Finally, both patatin enzymes catalysed detectable levels of butyl esters in mixtures containing 0.5, or 5.0% (v/v) butan-1-ol (results not shown).

DISCUSSION

A single isoform of the potato tuber protein, patatin, was produced and expressed from a baculovirus expression vector in cultured insect cells and subsequently assayed directly for its lipid-hydrolysing and acyl-transferring activities. The baculovirus expression vector utilizes the AcNPV polyhedrin promoter [26] modified for the insertion of foreign genes. This allows expression of prokaryotic or eukaryotic genes to produce fused or non-fused recombinant proteins [27]. One of the major advantages of this invertebrate-virus expression vector over bacterial, yeast and mammalian expression systems is the very abundant expression of eukaryotic recombinant proteins [12,28,29] from foreign genes. Additionally, the recombinant proteins are *N*-linked glycosylated [20,30,32], secreted [12,31] and retain functions, including antigenicity, similar to their '*in vivo*'-produced counterparts [12,20,31–34]. The virus is not pathogenic to vertebrates or plants, and the insect system does not require transformed cells as do the mammalian expression systems [35,36].

Our results clearly show that the patatin isoform patatin_{GM01} has lipid acyl hydrolase and acyltransferase activities. The enzymic properties of patatin_{GM01} are similar to that of potato-tuber-tissue lipid acyl hydrolase previously described [8]. This includes the biphasic nature of *p*-nitrophenyl acyl ester carbon chain length in influencing hydrolysis. Our finding that a single isoform of patatin also exhibits a biphasic optimum of activity suggests that the chain-length specificity seen by Galliard [8] is an intrinsic property of an individual enzyme species rather than being due to a mixture of different species.

Patatin_{tuber} and patatin_{GM01} displayed maximum activity with the 10-carbon *p*-nitrophenyl acyl esters, although most plant lipids contain fatty acids with carbon-chain lengths greater than 14. However, seed lipids from certain plants contain acyl carbon chains with less than the usual minimum number of carbon atoms [37]. The presence of 10-carbon fatty acids in tuber tissue has not been documented. In general, the activities of our enzymes were influenced by pH and substrate concentration in a similar fashion to that previously reported [8,38].

Our data also contain significant differences from previous findings. Galliard [8] reported that potato-tuber-tissue lipid acyl hydrolase had comparable specific activities with phosphatidylcholine and galactolipids [8]. We obtained similar specific activities with phosphatidylcholine, but found that the specific activity of patatin_{GM01} with galactolipids was 30-fold less when assayed at either pH 8.2 or pH 5.0. Furthermore, Galliard [8] noted substantial activity with diolein, but neither patatin_{GM01} nor patatin_{tuber} had detectable levels of activity with this substrate under our assay conditions. We also were not able to show Triton X-100 activation to the extent he reported. Whether these differences are due to contaminating activities in his preparations, such as the short-chain esterase activity noted by Racusen [7], or to differences between potato cultivars is not known. There is a precedent for difference in substrate specificity in the potato cultivar Désirée, which contains normal amounts of patatin, but lacks laurate (dodecanoate) esterase activity [39].

The physiological role of the enzymic activities associated with patatin is subject to speculation, but we

think that it may be involved in tuber tissue response to wounding. The tuber lipid acyl hydrolase is normally sequestered in lysosomes [38] and thus biologically inactive, but upon mechanical damage or pathogen invasion the enzyme would be released. In addition to providing substrates for production of toxic fatty acid derivatives and waxes that could inhibit the invasion of pathogens, patatin might also be involved in phytoalexin production, since it would release arachidonic acid, which is a known potent elicitor of phytoalexins [40].

We are grateful to Dr. Herman Wenzler for his help in manuscript preparation and to Dr. Randall Wood for assistance with lipid analysis and for helpful suggestions. This work was supported by grant 85-CRCR-1-1792 from the U.S. Department of Agriculture competitive grants program and funds from the Texas Agricultural Experiment Station.

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Received 7 July 1987/20 November 1987; accepted 20 January 1988