

From: THE METABOLISM, STRUCTURE, AND FUNCTION
OF PLANT LIPIDS
Edited by Paul K. Stumpf, J. Brian Mudd,
and W. David Nes
(Plenum Publishing Corporation, 1987)

GLYCOPROTEIN NATURE OF LIPOLYTIC ACYL HYDROLASES IN POTATO

TUBERS AND LEAVES

INTRODUCTION

The leaves and tubers of potatoes contain high levels of lipolytic acyl hydrolase activities (1,2,3). These enzymes are capable of hydrolyzing all endogenous phospholipids and galactolipids. It was recently reported that all of the lipolytic acyl hydrolase activity in potato tubers is associated with a glycoprotein fraction called "patatin" which comprises about 30% of the soluble protein in tubers (4). This study was undertaken in order to verify whether this finding was valid for other varieties of potatoes (in ref 4 Kennebec was the only variety studied). In addition, experiments were also conducted to determine whether the lipolytic enzymes in potato leaves were also associated with patatin or other glycoproteins.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* c.v. Russet Burbank) plants were grown from seed tubers as previously described (3). Leaves (5 g) were homogenized in a chilled mortar with a buffer (20 ml) containing 25 mM potassium phosphate (pH 7.0) and 2mM sodium metabisulphite. Tubers (40 g) were homogenized in a Waring Blendor with the above buffer (80 ml). Homogenates from leaves or tubers were filtered through 2 layers of cheesecloth and centrifuged at 100,000 g for 50 min. An aliquot (5 ml) of the 100,000 g supernatant fraction was desalted on a Sephadex G-50 column (1.5x30 cm) eluted with 25 mM potassium phosphate (pH 7.0). This desalted fraction was applied to a DEAE cellulose (DE-52) column (1.5x4 cm) rinsed with 3 bed volumes of the above buffer, 2 bed volumes of 0.25 M NaCl, and finally, 2 bed volumes of 0.5 M NaCl. The fraction eluted with 0.25 M NaCl was applied to a Con A Sepharose column (1x6 cm) which was eluted with 3 bed volumes of the above buffer, 2 bed volumes of 20 mM α -methyl glucose (to yield patatin fraction as described in ref 4), and finally 2 bed volumes of 300 mM α -methyl glucose. Small samples of each of the above fractions were retained for accurate book-keeping of enzyme yields and recoveries. The rates of hydrolysis of PNP-laurate, 4-MU-laurate, and C₆-NDB-PC were assayed as described (3,4,5).

RESULTS AND DISCUSSION

Patatin was prepared as previously described (4), and total protein

and three different lipolytic enzyme assays were measured in each of the steps of its preparation (Table 1). Two additional steps were added which did not appear in the original report of lipolytic acyl hydrolase in patatin (4). The 0.5 M NaCl eluate and the 300 mM α -methyl glucose eluate were included to remove protein which may have still been retained on the columns after elution with 0.25 M CaCl and 20 mM α -methyl glucose (Megl), respectively. Most of the PNP-laurate hydrolase activity was retained on the Con A column as previously reported, but only about half of the activity was eluted in the patatin (20 mM Megl eluate) fraction and the rest was eluted with 300 mM Megl. In the previous report essentially all of the lipolytic acyl hydrolase (PNP laurate hydrolase) activity was associated with patatin (4). A possible reason for the differences in this study and the previous one may be due to the fact that different cultivars of potatoes were used (Russet Burbank here versus Kennebec). We are currently investigating this possibility, especially in light of our previous report of substantial varietal differences in the total levels of lipolytic enzymes (6). In this experiment and in the next an appreciable loss of enzyme activity occurred with each chromatographic step, perhaps due to removal of a cofactor, proteolytic degradation, or other problems caused by instability of the enzymes. The distribution of C_6 -NBD-PC hydrolase in the various fractions was very similar to that of PNP-laur hydrolase, except that more activity was found in the 0.5 M NaCl DEAE eluate. When 4-methyl umbelliferyl laurate hydrolase activity was assayed in the various fractions 38% of the activity was apparently nonglycosylated (Con A Sepharose effluent). It therefore appears that the three different substrates were hydrolyzed at different rates by lipolytic enzymes which differed according to charge and degree of glycosylation. This is consistent with the previous report (5, also with Russet tubers) that three different lipolytic enzymes with different substrate specificities could be separated on DEAE cellulose (peaks at 0, 0.15, and 0.25 M CaCl). The conclusion of this experiment is that most of the lipolytic enzymes in potato tubers are glycoproteins as recently reported (4), but not all of them appear to be associated with patatin (at least in Russet Burbank).

In the second experiment (Table 2) patatin was purified from potato

Table 1. Phospholipase activity in the various stages of purification of the glycoprotein "patatin" from potato tubers (c.v. Russet Burbank).

Fraction	protein (mg/fraction)	esterase activity		phospholipase activity
		PNP-Laur units ^a	4-MU-Laur units ^a	C_6 -NBD-PC units ^a
100,000 g supernatant	20.4	17.8	7.69	8.84
Sephadex G-50 void	5.89	12.8	5.29	7.35
DEAE cellulose				
Effluent	1.35	0	0	0
Eluate (0.25 M NaCl) (put on Con A Seph)	3.67	10.6	4.13	6.00
Eluate (0.50 M NaCl)	0.16	0.49	0	1.47
Con A Sepharose				
Effluent	1.26	0.30	1.10	0
Eluate (20 mM Megl) (= patatin)	1.08	3.41	0.82	2.00
Eluate (300 mM Megl)	0.87	3.79	1.01	2.42

Table 2. Phospholipase activity in the various stages of purification of the glycoprotein "patatin" from potato leaves (c.v. Russet Burbank).

Fraction	protein (mg/fraction)	esterase activity 4-MU-Laur units ^a	phospholipase activity C ₆ -NBD-PC units x 10 ⁻³
100,000 g super	30.5	87.7	19.8
Sephadex G-50 void	14.6	42.6	16.5
DEAE cellulose			
Effluent	1.72	0	0
Eluate (0.25 M NaCl) (put on Con A Seph)	10.7	29.9	5.59
Eluate (0.50 M NaCl)	1.03	0	1.24
Con A Sepharose			
Effluent	10.19	30.1	2.01
eluate (20 mM Meglc) (= patatin)	0.23	0	0.65
Eluate (300 mM Meglc)	0.03	0	0.13

^a enzyme activity = $\mu\text{mol min}^{-1} \text{ fraction}^{-1}$

leaves and two different lipolytic enzymes were measured in the various steps. Since PNP-laurate hydrolase was barely detectable it was not reported. All of the 4-MU-laurate hydrolase activity occurred in the 0.25 M NaCl DEAE eluate and the Con A Sepharose effluent, indicating that it was not associated with patatin. Much lower levels of C₆-NBD-PC hydrolase activity were detected. Although most of this activity also was found in the Con A Sepharose effluent, significant levels were also found in the 0.5 M NaCl DEAE eluate, and the patatin fractions. This experiment indicates that unlike potato tubers, very little of the lipolytic activity in potato leaves is associated with patatin or other glycoproteins which bind to Con A.

In a final experiment we investigated the possible occurrence of other common (nonlipolytic) hydrolases in the patatin fraction from potato tubers. High levels of acid phosphatase and N-acetyl glucosaminidase activities were detected (data not shown). The occurrence of at least 3 different enzyme activities in the patatin fraction causes one to question whether the 6-10 isoforms (ionic forms) which comprise the patatin fraction actually have very much in common (4).

REFERENCES

1. T. Galliard, The enzymatic deacylation of phospholipids and galactolipids in plants. Purification and properties of a lipolytic acyl-hydrolase from potato tubers, *Biochem. J.* 121:379 (1971).
2. H. Matsuda and O. Hirayama, Purification and properties of a lipolytic acyl hydrolase from potato leaves, *Biochem. Biophys. Acta.* 573:155 (1979).
3. R. A. Moreau, Regulation of phospholipase activity in potato leaves by protein phosphorylation-dephosphorylation, *Plant Science* (in press).
4. D. Racusen, Lipid acyl hydrolase of patatin, *Can. J. Bot.* 62:1640 (1984).
5. E. P. Hasson and G. G. Laties, Separation and characterization of potato lipid acylhydrolases, *Plant Physiol.* 57:142 (1976).
6. R. A. Moreau, Membrane-degrading enzymes in the tubers of various cultivars of *Solanum tuberosum*, *J. Ag. Food. Chem.* 33:36 (1985).