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PHOSPHOLIPASE, GALACTOLIPASE AND ACYL TRANSFERASE ACTIVITIES OF A LIPOLYTIC ENZYME FROM POTATO

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; lipolytic acyl hydrolase; phospholipases; galactolipase; acyl transferase.

Abstract—Characterization of reaction products showed that an enzyme (lipolytic acyl hydrolase) isolated from potato tubers could act on endogenous substrates as a galactolipase (E.C. 3.1.1.26), lysophospholipase (E.C. 3.1.1.5) or a 'phospholipase B' but not as a lipase (E.C. 3.1.1.3). The affinity of the enzyme for methanol as acyl acceptor (acyl transferase activity) was higher than its affinity for water (acyl hydrolase activity). The nomenclature of acyl hydrolases in plants is discussed.

INTRODUCTION

THE NOMENCLATURE of plant enzymes that catalyse the deacylation of lipids is not rigorous. Such enzymes have been variously described as lipases, phospholipases, galactolipases and esterases, frequently based on results with a limited range of substrates (for reviews see Refs 1–4). Unequivocal characterization of specific acyl hydrolases, with the exception of seed lipases (glycerol ester hydrolase E.C. 3.1.1.3) is rare in literature on plant enzymes. For example, the sulpholipase activity of plants was associated with a phospholipid deacylation activity although not with galactolipid deacylation.[5]

The characterization of an enzyme with broad specificity for polar lipids (phospholipids, galactolipids, mono- and di-glycerides) as well as some *p*-nitrophenyl-, naphthyl- and methyl esters of long chain fatty acids, but inactive on triglycerides, wax esters and sterol esters,[6] demonstrated the need for a re-examination of the specificities and nomenclature of this group of hydrolytic enzymes. A further complication is the possibility of reverse hydrolysis (i.e. acyl ester formation) or acyl transfer reactions catalysed by acyl hydrolases.[7] This communication illustrates the fact that, under appropriate conditions, an enzyme from potato tuber acting on endogenous substrates may be described as a phospholipase, a galactolipase or an acyl transferase.

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RESULTS

Phospholipase activity

Previous studies have established that one enzyme is responsible for the liberation of free fatty acids from mono- and diacyl phospholipids and from mono- and di-galactosyl diglycerides.[6,8] However, the non-lipid reaction products were not examined and the possibility existed that the hydrolysis could involve the polar groups and the lipids in a more complex mechanism unlike phospholipase B or galactolipase action.

Using a partially purified enzyme preparation from potato tubers, the products obtained with phosphatidylcholine, lysophosphatidylcholine, mono- and di-galactosyl diglycerides as substrates were examined. The enzyme concentrations used were selected to give approximately 50% deacylation of substrate in 10 min under the conditions used. Incubations of 10 and 60 min duration were performed, after which the chloroform- and water-soluble products were separated and analysed chromatographically.

TABLE 1. HYDROLYSIS PRODUCTS FROM PHOSPHOLIPIDS AND GALACTOLIPIDS BY THE ACTION OF LIPOLYTIC ACYL HYDROLASE

Phosphatidylcholine	Free fatty acids + glycerylphosphorylcholine
Lysophosphatidylcholine	Free fatty acids + glycerylphosphorylcholine
Monogalactosyldiglyceride	Free fatty acids + monogalactosylglycerol + monogalactosyl monoglyceride*
Digalactosyldiglyceride	Free fatty acids + digalactosylglycerol

* Minor product.

The results (Table 1) showed that the phospholipid substrates were hydrolysed to the corresponding deacylated forms and that none of the following possible water-soluble hydrolysis products was detected even after a 60 min incubation: glycerol, glycerophosphate, phosphorylcholine, choline or inorganic phosphate. Also, no monoacyl intermediate (indicative of phospholipases A_1 or A_2) was found in the deacylation products of phosphatidylcholine and no evidence of phosphatidic acid or diglyceride (indicative of phospholipases D and C respectively) was obtained.

In the absence of additional surface active agents the enzyme is much more active on lysophospholipids than on the diacyl analogues[6] and this fact, together with the above results, shows that the enzyme satisfies the criteria of lysophospholipase (E.C. 3.1.1.5). In the presence of a surface active agent (e.g. Triton X100 or linoleic acid) phosphatidylcholine is hydrolysed to glycerylphosphorylcholine without accumulation of the lyso-intermediate.[6]

Galactolipase activity

The galactolipid substrates similarly gave only the corresponding deacylated products on incubation with the enzyme (Table 1). No glycerol, galactose or, in the case of digalactosyldiglyceride, any digalactosylmonoglyceride was detected. However, as observed previously,[8] a small amount of monogalactosyl monoglyceride was detected as an intermediate in the deacylation of monogalactosyl diglyceride. The reaction products obtained from the galactolipids, together with previous results,[6,8] show that the enzyme acts as a galactolipase (E.C. 3.1.1.26).[9] The detection of a monoacyl derivative in the hydrolysis products of monogalactosyl diglyceride indicates that a stepwise deacylation mechanism is involved.

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Acyl transferase activity

The earlier work had demonstrated that the lipolytic acyl hydrolase enzyme could catalyse acyl transfer reactions between a range of lipid substrates and primary alcohol acceptors.[6,8] Further study of this phenomenon has shown that, under appropriate conditions, the acyl transferase activity of the enzyme can predominate over the hydrolytic activity.

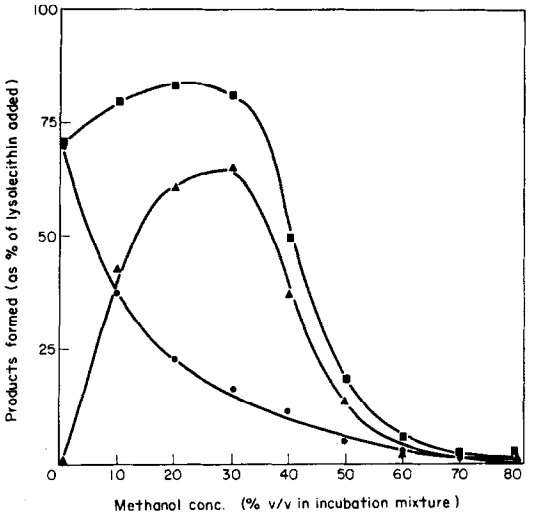

FIG. 1. EFFECT OF METHANOL CONCENTRATION ON ACYL TRANSFERASE ACTIVITY OF LIPOLYTIC ACYL HYDROLASE.
Amounts of free fatty acids (●——●) and fatty acid methyl esters formed (▲——▲) or lysophosphatidylcholine deacylated (■——■) in 10 min incubations at 25° are given as % of substrate (lysophosphatidylcholine) added. Experimental details are given in text.

Figure 1 illustrates the effect of methanol concentration on the hydrolytic and acyl transferase activities of the enzyme. With lysophosphatidylcholine as substrate, methanol concentrations above 30% (v/v) cause a marked inhibition in the deacylation but below this concentration the deacylation is not inhibited and may be slightly stimulated. The proportion of fatty acid methyl ester to free fatty acid increases to a maximum of 4:1 at a methanol concentration of 30% (v/v). Inspection of Fig. 1 shows that, at 10, 20 and 30% methanol concentrations, the affinity of the enzyme for methanol as acyl acceptor is approximately 10-fold that for water.

DISCUSSION

The above results have illustrated the possible ambiguities inherent in nomenclature of enzymes with broad specificities. This problem is due in part to possible assumption of specificity based on recognized assay methods for established enzymes. Unpublished work in this laboratory has shown that the lipolytic acyl hydrolase activity occurs in many plants but that potato tubers contain higher activities than other tissues so far studied. For example, our studies with leaves of *Phaseolus vulgaris* have shown that the enzyme previously identified as galactolipase (E.C. 3.1.1.26)[9] is in fact a less specific enzyme and it is probably identical with the lipolytic acyl hydrolase of potato tubers (Helmsing, personal communication).

The term phospholipase "B" was originally applied to an enzyme that removed both acyl groups from a diacyl phospholipid although available evidence[10] indicated that this activity could be ascribed to a mixture of two enzymes (phospholipases A_1, E.C. 3.1.1.32 and A_2, E.C. 3.1.1.4) each specific for one of the two acyl ester bonds. Subsequently, phospholipase B has been defined as an alternative name for lysophospholipase (E.C. 3.1.1.5).[10,12] However, an enzyme obtained from barley and described as phospholipase B[13] is very similar to the lipolytic acyl hydrolase from potato in that both enzymes remove both acyl groups from phosphatidylcholine in reactions activated by free fatty acids.

However, the lipolytic acyl hydrolase from potato (and the "galactolipase" from *P. vulgaris* leaves[14]) are most active on monoglycerides (c.f. monoacyl glycerol hydrolase, E.C. 3.1.1.23)[12] and lysophospholipids (c.f. lysophospholipase, E.C. 3.1.1.5).[11,12] The potato enzyme hydrolyses diglycerides (c.f. diacyl glycerol lipase, E.C. 3.1.1.34)[12] and other esterified forms of long chain fatty acids but does not hydrolyse the non-polar triglycerides (c.f. lipase, i.e. triacyl glycerol acyl hydrolase, E.C. 3.1.1.3), sterol esters (c.f. sterol ester hydrolase, E.C. 3.1.1.13) or wax esters. With reference to the acyl transfer activity of the enzyme, it should be noted that, although it is conventionally named as an acyl hydrolase, its greater affinity for an alcohol than for water as acyl acceptor merits a definition of an acyl transferase.

EXPERIMENTAL

Materials. Potato tubers (*Solanum tuberosum* var. Majestic) were obtained at commercial harvest and stored at 5° until used. Phosphatidylcholine (from egg yolk) and glyceryl phosphorylcholine were obtained from Sigma Chemical Co. Lysophosphatidylcholine and ^{14}C-lysophosphatidylcholine (labelled with ^{14}C in the fatty acyl moiety) were prepared as previously described.[15] Mono- and di-galactosyl diglycerides and their deacylation products were obtained from spinach leaf lipids.[16]

Preparation of lipolytic acyl hydrolase enzyme. For phospholipase and galactolipase activity studies a partially purified preparation of the enzyme from potato tubers[6] in 2M $(NH_4)_2SO_4$ was passed through a column of Sephadex G25, equilibrated and washed with H_2O, to remove salts which interfered with subsequent chromatography. The salt-free enzyme solution was frozen in small aliquots until used. For acyl transferase studies we used a crude 15000 *g* supernatant fraction from tubers homogenized in 0·05 M potassium buffer, pH 6·0, containing 100 μM mercaptobenzathiazole.

Enzyme incubations and assays. For phospholipase and galactolipase studies no buffers were used. Substrates were sonicated in water;[6] optimal substrate concentrations[6] as used were 3 mM phospholipids or 0·8 mM galactolipids; linoleic acid (3 mM) was added as activator to systems containing phosphatidylcholine.[17] Amounts of enzyme used were calculated to give *ca* 50% breakdown of substrate in 10 min incubation (i.e. for phosphatidylcholine, lysophosphatidylcholine, monogalactosyl diglyceride and digalactosyl diglyceride, 680, 120, 85 and 150 μg of protein respectively). Initial pH values were between 5·0 and 6·0, mixtures being made up to 2·4 ml with distilled H_2O. After incubation at 25° for 10 min, reactions were stopped by heating at 100° for 3 min. Chloroform-soluble and aqueous methanol-soluble products were separated.[16] Chloroform-soluble products were applied to thin layers (0·25 mm) of silica gel G. Aq. methanol phases were taken to dryness *in vacuo*, residues taken up in distilled H_2O and suitable amounts applied to acid-washed Whatman No. 1 PC. For acyl transferase studies, incubation mixtures contained ^{14}C-lysophosphatidylcholine (2 μmol, 0·1 μCi); 0·05 M sodium acetate buffer, pH 5·5; enzyme (100 μg protein); methanol (0–0·8 ml as indicated in Fig. 1) and water to make a final vol of 1·0 ml. After 10 min incubation at 25°, reactions were stopped and extracted as above. Aliquots of the chloroform-soluble products (0·05 μCi) were applied in 1 cm bands to silica gel G TLC plates.

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Chromatography. Non-polar chloroform-soluble products from enzyme incubations were separated by TLC in petrol. (B.P. 60-80°)–Et_2O–HOAc (60:40:1). Polar products were separated on TLC plates developed in CHCl–MeOH–HOAc–H_2O (70:30:20:4). Lipids were visualized with I_2 vapour. For assay of acyl transfer activity ^{14}C-labelled products separated in the non-polar TLC system were located with a radiochromatogram scanner (Panax Equipment Ltd.) and appropriate areas of the gel (containing lysophosphatidylcholine, free fatty acid or fatty acid methyl esters) were scraped into scintillation fluid for liquid scintillation counting.[15] H_2O-soluble deacylation products were separated by descending paper chromatography in *n*-PrOH–NH_4OH (0·88 sp. gr.)–H_2O (6:3:1).[18] The following authentic standards were chromatographed in the same system (R_f values in brackets): glycerol (0·69); monogalactosyl glycerol (0·49); galactose (0·48); choline (0·45); glyceryl phosphorylcholine (0·42); digalactosyl glycerol (0·30); sodium glycerophosphate (0·26); phosphatidylcholine (0·23); KH_2PO_4 (0·14). Conventional detection methods were used for compounds containing polyhydroxy,[19] phosphate[20] and choline[21] groups.

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