Lipid Catabolism: Lipid Degradation

Lipase activity in germinating sunflower seedlings
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Abstract
Studying lipase in germinating sunflower seedlings, we looked for an activator of the lipolytic activity. In the presence of 1.25 mM ATP, the enzyme activity increased 2-fold. Lipid-body lipase solubilization was realized using two detergents: Tween 80 and CHAPS. Lipolytic activity was increased 10-fold in the presence of 2% (w/v) CHAPS, showing the probable ‘complexity’ of the enzyme. Looking for the possible lipolytic activity of the 10000 g pellet we detected the presence of the enzyme. The pellet extract was mixed, in a range of concentrations, with the oil-body fraction. The resulting lipolytic activity was 4-fold higher. These results give clues as to the subcellular distribution of lipase and its intracellular transport.

Introduction
Lipases are triacylglycerol acylhydrolases (EC 3.1.1.3.) involved in the first steps of lipid catabolism. The interest in these enzymes has increased with their use in food technology, medicine and industrial chemistry [1,2]. Plant lipases, contrary to those of micro-organisms and animals, are not well known. Because of the water-insoluble substrate, great confusion has surrounded studies of these enzymes, and clarification of true lipase has only been recently done [3]. Here we try to contribute to a better understanding of the mechanism of lipolytic activity in germinating sunflower seedlings.

Experimental
Oil-body fraction: pellet fraction
We used 3-day-old cotyledons from germinating sunflower seedlings (Helianthus annuus L. var Albena). The oil bodies were extracted using the methods of [4] adapted for the sunflower [5]. The resulting 10000 g pellet was used as the ‘pellet fraction’, the activity of which was studied comparatively. All operations were performed at 0–4 °C.

Table I
The effect of ATP and GTP on lipolytic activity in oil bodies

<table>
<thead>
<tr>
<th>Nucleotide added</th>
<th>Specific activity (pkat/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>40.8</td>
</tr>
<tr>
<td>ATP (1.25 mM)</td>
<td>114</td>
</tr>
<tr>
<td>GTP (1.25 mM)</td>
<td>52</td>
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</tbody>
</table>

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Oil-body lipase solubilization
Two detergents were selected: a non-ionic detergent, Tween 80, and a zwitterionic one, CHAPS. The oil-body membrane was resuspended in 0.15 M Tris/HCl buffer (pH 7.5). To the lipid-body fraction, detergents were added from 0.25 to 2% (w/v) for CHAPS and from 0.65 to 3.25% (w/v) for Tween 80. The mixture was stirred at 4 °C for 30 min and then centrifuged at 10000 g for 20 min. Activity was measured in the supernatant.

Lipase activity
A colorimetric method, adapted from [6], was used to measure lipolytic activity. The substrate, a purified sunflower oil, was emulsified in arabic gum (10%). The reaction was carried out at 30 °C and a neutral pH value was retained for tests.

Results and discussion
As seeds contain important amounts of triacylglycerols in subcellular droplets called oil bodies or lipid bodies [7], we first investigated the presence of lipolytic activity in this fraction [5]. During lipase-purification steps, we observed after (NH₄)₂SO₄ treatment of the crude extract (52 g/l) that lipolytic activity decreased rapidly during dialysis. After 90 min of dialysis 25% of the lipolytic activity was lost (results not shown). This result suggested to us the possible loss of a ligand or a cofactor which could be essential for the enzymatic activity.
Lipase solubilization by detergents

Oil-body protein (0.71 mg/ml) was stirred with diverse critical micelle concentrations of each detergent. CHAPS is well known to solubilize membrane proteins [10,11]. This non-denaturating detergent will be used for further studies on microsomes.

Effect of nucleotides on lipolytic activity

We tested the possible interaction between ATP, GTP and lipase. The enzyme was incubated in the presence of nucleotide in the concentration range of 1.25–5 mM. Only the lowest concentration had a significant effect. Table 1 shows that the lipolytic activity increased 2-fold in the presence of 1.25 mM ATP, and that the stimulation was not significant in the presence of 1.25 mM GTP.

Oil-body lipase solubilization

Figure 1 shows the effect of increasing Tween 80 and CHAPS concentration on lipase solubilization. This was optimal with 2% (w/v) CHAPS, where about 67% of total proteins were solubilized and the enzymic activity was increased 10-fold (specific activity, 1200 pkat/mg). This is in contrast to Tween 80, for which lipase activity was optimal at the detergent’s critical micelle concentration (1.3%, w/v), with a specific activity corresponding to 166.5 pkat/mg and where about 67% of total proteins were also solubilized.

Figure 3

Lipolytic activity of the enzymic mixture on the rate of lipolysis

This result proves the presence of an activator in the pellet fraction. Could the sunflower lipolytic system be equivalent to the acid castor bean lipase [14]? Further work will explore microsomes and detect lipase using CHAPS to solubilize this enzyme.
The 10,000 g pellet’s lipolytic activity
The investigation of the lipolytic activity in the 10,000 g pellet fraction is shown in Figure 2. A linear relationship was obtained between the amount of pellet extract added and enzymic response.

The conjugated lipolytic activity of lipid body plus pellet
We mixed the pellet extract with the oil-body fraction in the presence of free Ca²⁺ (200 μM). We observed (Figure 3) that the resulting activity was 4-fold higher with the addition of 130 μg of protein pellet. This corresponds to a ratio of oil body to pellet of 1:3.

Conclusion
The subcellular distribution of lipase could be regulated. ATP and calcium seemed to be required for lipolytic activity in germinating sunflower seedlings. Is the studied lipolytic system so far from that of animals?

References
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Assaying Arabidopsis lipase activity
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Abstract
A low lipase activity from a crude extract of Arabidopsis seedlings was assayed using three sensitive methods (radio labelled triacylglycerols, commercial resorufin ester and triacylglycerols containing the naturally fluorescent parinaric acid as substrates). The specific activity of the extract was found to be similar using the three methods. However, the plant lipase activity measured using the radioactivity and the fluorescence assays could be abolished by heating the extract, contrary to the apparent activity measured using the commercial colorimetric assay. Unlike the radioactivity assay, the fluorescence assay can be monitored continuously. The parinaric acid-based method is therefore the only one to provide a sensitive, specific and continuous assay.

Introduction
True lipases (EC 3.1.1.3) are enzymes that catalyse the hydrolysis of the carboxyl ester bonds of long-chain acylglycerols at an oil/water interface [1]. These enzymes have been well characterized in mammals and micro-organisms [2]. By contrast, very little is known about plant lipases. This is mainly due to the very low abundance of these proteins, which makes them difficult to purify in amounts sufficient to get access to amino acid sequence information. Therefore, no plant true lipase has ever been cloned.

During post-germination of an oilseed plant such as Arabidopsis thaliana, the growth of the