LIPOLYTIC ENZYMES IN GROWING SEEDS OF THE COTTON PLANT

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The enzymatic cleavage of lipids and phospholipids of oil-containing seeds is a necessary step of the early stage of their development. In order to study the optimum conditions for extracting lipolytic enzymes, we have investigated the changes in the amount and enzymatic activity of the phospholipase D and lipase in the sprouting of cotton seeds.

The seeds were sprouted in the light at 20°C on carefully washed sand. Every day 50 ml of distilled water was added per 100 g of seeds. Samples were taken and they were defatted five times by triturating them in a mortar with cold acetone and then with diethyl ether. A portion of the acetone powder obtained in this way was triturated with an equal volume of ground glass in a mortar containing water (double distilled) in a ratio of 1 : 10. The suspension was centrifuged for 30 min (18,000 × g, 2°C).

The activity of the lipase at pH 8.75 and the activity of the phospholipase D at pH 5.6 were measured by published methods [1, 2]. In parallel with the measurement of the activity, the content of free choline [3], the amount of protein extracted, the total amount of lipids, and the change in the turbidity of the suspension were measured (Table 1). It can be seen from Table 1 that the lipase activity gradually increases, reaching a maximum on the fourth day after the beginning of sprouting. A fall in the total content of lipids correlates with the increase in lipase activity. Conversely, the activity of the phospholipase D does not undergo substantial changes, although the level of free choline rises, this rise correlating with the change in the lipase activity. The question of how far these facts are interconnected remains open. With an increase in the lipase activity, the light scattering of the system decreases, possibly as a result of the passage of the lipase into a more soluble form. The decrease in the amount of extractable protein observed during sprouting, which is connected with the synthesis of a highly active proteinase in sprouting cotton seeds [4] also possibly plays a part in this. How far the increase in the activity of the lipase is connected with the synthesis of the proteolytic enzymes is difficult to establish at the present time.

TABLE 1

Time of growth, days	Lipase activity*	Contents of lipids, %	Phospholipase D activity*	Choline content, mM	Optical density	Protein extracted, mg
1	5,1	30	9,8	$3{,}4 \cdot 10^{-4}$	1,05	256
2	8,1	28	10,5	$6{,}9 \cdot 10^{-4}$	0,825	158
3	16,8	26	10,0	$15{,}5 \cdot 16^{-4}$	0,487	143
4	27,6	20	10,8	$18{,}0 \cdot 10^{-4}$	0,280	108
5	25,8	16,5	11,0	$12{,}1 \cdot 10^{-4}$	0,350	118
6	15,6	—	—	—	—	—
7	13,6	15	11,0	$7{,}1 \cdot 10^{-4}$	0,660	167
8	10,8	—	9,5	$5{,}0 \cdot 10^{-4}$	0,750	180

*The activities of the enzymes are expressed in millimicromoles of substrate hydrolyzed by 1 mg of protein in 1 min at 25°C.

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