

## THE PHOSPHOLIPASE ACTIVITY OF THE FUNGUS

*Rhizopus microsporus* UzLT-1

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We have previously [1, 2] reported the presence in the culture liquid of the fungus *Rhizopus microsporus* of extracellular and intracellular lipases (E.C. 3.1.1.3 - glycerol ester hydrolases).

In the present communication we give information on the phospholipase activity of the total protein obtained from the culture liquid. As the substrate we used phosphatidylcholine (PC) isolated by a published method [3]. Enzymatic hydrolysis was carried out at pH 10.2 and 8.4, the amount of total protein being 10 and 100 mg. In all cases, we took the same amount of PC - 100 mg, dissolved in 20 ml of ether, 1.5 ml of 0.1 M Tris buffer, and 1 ml of 0.1 M CaCl<sub>2</sub>. The time of complete hydrolysis was 2-16 h, the reaction being most active with the conditions 100 mg of total protein at pH 8.4. The completeness of hydrolysis was checked by TLC on silica gel in the chloroform-methanol-25% ammonia (70:30:5) system.

The reaction products were separated as described in the literature [3]. The free fatty acids and those obtained after the alkaline hydrolysis of the lyso-PC were methylated by diazomethane. Analysis of the fatty acid methyl esters was carried out on a UKh-2 chromatograph (column 0.4 × 200 cm, 17% of PEGS on Celite 545, 80-100 mesh, with helium as the carrier gas).

The results of the analysis of the fatty acids split off after enzymatic hydrolysis showed that the total protein contained an enzyme that splits off the fatty acids in position 1 of the glyceride moiety of PC with strict specificity, which is in harmony with the results for the fatty acid composition of the lyso-PC obtained after enzymatic hydrolysis with phospholipase A<sub>2</sub> [3]. Thus, it may be assumed that the total protein contains the enzyme phospholipase A<sub>1</sub>.

This is the first time that a phospholipase A has been detected in the protein of the fungus *R. microsporus*. It can be used successfully for structural investigations of glycerophospholipids.

### LITERATURE CITED

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