A LIPASE OF THE FUNGUS Rhizopus microsporus, UzLT-1 – A GLYCOPROTEIN

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In recent years, the lipases (glycerol ester hydrolases, E.C. 3.1.1.3) of various microorganisms have been intensively studied. Some of them have been obtained in the highly purified state [1-3].

In the present paper we consider the results of a study of the nonprotein fraction of the lipase of the fungus Rhizopus microsporus, UzLT-1. We have described the purification of this enzyme previously [4].

To estimate the homogeneity of the enzyme obtained we used sedimentation analysis and disk electrophoresis. On ultracentrifugation in 0.01 M phosphate buffer (pH 7.8), one symmetrical peak with a sedimentation coefficient of 2.8 S, corresponding to a molecular weight of 40,000 was found in the purified enzyme. It must be mentioned that this preparation is unstable on storage. Thus, when the homogeneous preparation was stored under refrigerator conditions for a year, the appearance of a second more cathodic component with a molecular weight of 30,000 took place. The separation of the two components of the lipase was performed by a column of Sephadex G-75 with phosphate buffer, pH 7.8. The native component of the enzyme contains carbohydrates, as was shown by disc electrophoresis and staining with the Schiff reagent [5].

The carbohydrate fraction is readily separated from the protein fraction of the enzyme on heating, on precipitating the proteins with trichloroacetic acid, and also by acidifying an enzyme solution with a 1 N solution of hydrochloric acid to pH 3.0, which permits the assumption of a noncovalent linkage of the sugars with the protein fraction.

The amount of carbohydrates in a homogenous preparation of the lipase was 8%. Paper chromatography showed the presence in a hydrolyzate of arabinose, xylose, and galactose in a ratio of 4 : 2 : 1, respectively.

EXPERIMENTAL

Determination of the Protein. The amount of protein in solutions was determined by the biuret reaction and by the Warburg–Christian method [6].

Determination of the Activity of the Enzyme. The enzyme was obtained by the method described previously [4]. Its lipolytic activity was determined by a titrimetric method based on the determination of the free fatty acids formed as a result of the hydrolysis of lipids [3]. Before the activity determination, the enzyme solution was diluted to a concentration ensuring a decomposition of the substrate at which from 0.4 to 0.8 ml of 0.1 N KOH was consumed in the neutralization of the liberated fatty acids.

The lipolytic activity was expressed in milliliters of 0.1 N KOH consumed in the titration of the fatty acids formed in the hydrolysis of olive oil through the action of the enzyme under the conditions of the experiment.

Disk Electrophoresis in Polyacrylamide Gel was performed as described previously [7]. Electrophoresis was performed for 2 h (voltage 350-400 V, current strength not more than 5 mA per tube) at 4°C. After electrophoresis, the control gels were fixed in 7% acetic acid for 3 min and were then stained with a 0.25% solution of the dye Coomassie Blue in a mixture of methanol, water, and acetic acid (5 : 5 : 1). The dye residues were washed out with 7% acetic acid.

Revelation of the Glycoproteins in the Gel. After electrophoresis, the gels were kept in 7.5% acetic acid at 20°C for 1 h and in 0.2% periodic acid at 4°C for 1 h. Then the gels were incubated with the Schiff reagent (the

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Schiff reagent was prepared in the following way: to a 0.5% aqueous solution of pararosaniline (C.I. 42500) was added 1 g sodium bisulfite and 10 ml of 1 N HCl and the mixture was shaken for 3 h, and after the addition of 0.5 g of activated carbon it was filtered and stored in a well-stoppered glass bottle) at +4°C until the band had become red (1-2 h).

The neutral sugars were determined by the total orcinol method [8]. For the quantitative determination of the individual neutral sugars, samples of the enzymes (~10 mg) were hydrolyzed in 2 N HCl at 100°C for 2 h.

The acid was eliminated with Dowex 1 × 16 in the HCO_3^- form, and final desalting was performed by passing the supernatant liquid through a column (2.5 × 20 cm) of Dowex 50 × 8 in the H^+ form, after which it was subjected to paper chromatography in the butan-1-ol–pyridine–water (10 : 3 : 3) system [9], the solvent being run twice. The sugars were revealed with triphenyl-tetrazolium chloride in the following way [12]. The dried chromatogram was immersed for a short time in a solution of the revealing agent (200 mg of tetrazolium compound in 50 ml of methanol), dried in the air for 10 min, immersed in 0.5 M KOH in methanol, and dried again, then the spots were revealed by heating the sheet in an atmosphere saturated with water vapor at 65-70°C for 30 min. The spots were cut out and the dye was eluted with 5 ml of a mixture of methanol and acetic acid (8 : 1). The optical density of the solution was measured at 483 nm with deduction of the background given by the paper.

Markers were deposited on each chromatogram. All the operations were performed under low illumination.

Ultracentrifugation was performed in an ultracentrifuge (model 3170 MOM), using 0.01 M phosphate buffer at room temperature. The molecular weights were found by the method of unestablished equilibrium [13].

SUMMARY

The molecule of the lipase of the fungus Rhizopus microsporus UzLT-1 consists of protein and nonprotein moieties. The nonprotein moiety is formed by carbohydrates.

The carbohydrate moiety is readily separated from the protein moiety, which suggested a noncovalent bond of the sugars with the protein fraction.

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