LIPOPROTEIN LIPASE ACTIVITY OF THE FUNGUS *Rhizopus microsporus*

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A method is described for the isolation and purification of lipoprotein lipase activity from the culture liquid of the fungus *Rhizopus microsporus*. Some properties of the homogeneous enzyme have been studied: molecular weight 43,000, pI 3.7.

The fungus *Rhizopus microsporus* is known as an active producer of a lipase [1]. In the present paper we discuss the isolation, purification, and some physicochemical properties of an enzyme of this fungus that has not previously been studied, lipoprotein lipase (E.C. 3.1.1. 34).

In preliminary experiments, we studied the dependence of the precipitability of the enzyme protein on the pH of the culture liquid of the fungus *Rhizopus microsporus*. About 90% of the enzyme studied precipitates when the culture liquid is acidified to pH 4.2. The precipitate was collected by centrifugation, dissolved in the minimum amount of 0.1 M phosphate buffer, pH 7.8, and deposited on a column of Sephadex G-100 (Fig. 1).

The active fraction after separation on Sephadex was collected, concentrated by freeze-drying, dialyzed against $1 \cdot 10^{-3}$ M phosphate buffer, and deposited on a column of DEAE-Sephadex A-50 equilibrated with the same buffer. The protein was eluted first with the initial buffer and then with a linear gradient of increasing ionic strength of the buffer with added 0.5 M NaCl (Fig. 2a). As can be seen from Fig. 2, the activity under investigation was eluted in fractions 17-24, corresponding to a 0.08-0.1 M solution of NaCl. After concentration and dialysis, further purification was carried out on a column of CM-cellulose equilibrated with $1 \cdot 10^{-3}$ M acetate buffer, pH 5.6.

It can be seen from the elution graph (Fig. 2b) that the lipoprotein lipase activity is eluted completely by $5 \cdot 10^{-3}$ M acetate buffer, pH 5.6. The subsequent stage of purification, which led to a completely homogeneous separation, consisted in absorbing the active fractions after CM-cellulose, concentrating them, and subjecting them to gel filtration on a column of Sephadex C-100 (Fig. 3). The protein was eluted as a single symmetrical peak with a high lipoprotein lipase activity. Attempts at further purification of the enzyme on DEAE-cellulose, DEAE-Sephadex A-50, and SE-Sephadex, C-50 gave no effect. As a result, a 48-fold purification of the enzyme was achieved with a 23% activity yield.

The D_{280}/D_{260} ratio before and after gel filtration on Sephadex G-100 in the last stage remained constant, which indicates the chromatographic homogeneity of the enzyme. Homogeneity was also shown by analytical disc electrophoresis in 7.5% polyacrylamide gel (pH 8.9). The protein migrated with a R_f value of 0.45-0.47.

Thus, the scheme described above can be used to purify the lipoprotein lipase activity of the fungus *Rhizopus microsporus*.

The molecular weight of the homogeneous enzyme determined by the sedimentation method is about 43,000, as was also confirmed by gel filtration in a calibrated column of Sephadex G-100. The isoelectric points determined by the electric focusing method corresponds to pI 3.7.

EXPERIMENTAL

We have described the conditions of growing the producing agent previously [1, 2]. To precipitate the enzyme–protein complex we used 0.1 N HCl, the pH being monitored with the

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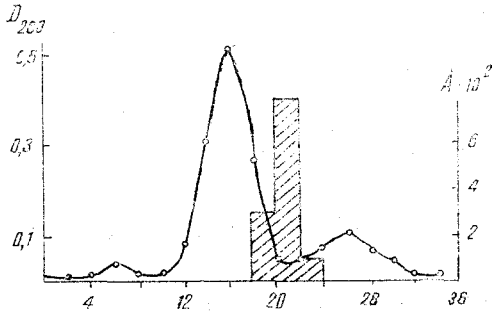

Fig. 1

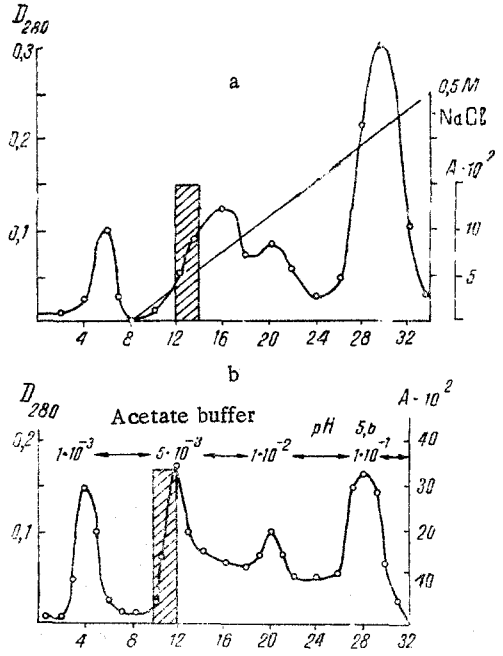

Fig. 2

Fig. 1. Gel chromatography of the total extracellular proteins of the fungus *Rhizopus microsporus* obtained by precipitating the culture liquid by acidification to pH 4.2 on Sephadex G-100 (here and below the hatched region indicates lipoprotein lipase activity. Column dimensions 2.2 × 85 cm. Eluant 0.1 M phosphate buffer, pH 7.4, rate of elution 14 ml/h).

Fig. 2. Chromatography of the active fraction: a) after Sephadex G-100 on DEAE-Sephadex A-50 (column dimensions 1.4 × 40 cm, rate of elution 12 ml/h, for conditions of elution see text); b) after DEAE-Sephadex A-50 on CM-cellulose (column dimensions 1.0 × 20 cm, rate of elution 12 ml/h, for conditions see text).

aid of a pH-340 pH-meter. The precipitate was collected by centrifugation in a TsLS-1 refrigerating centrifuge at 3000 rpm for 15 min.

Determination of Lipoprotein Lipase Activity. The substrate was prepared in the following way: 2.0 g of olive oil was mixed with 22.5 ml of a 2% solution of poly(vinyl alcohol) and the mixture was emulsified for 5 min in a homogenizer. A mixture of 1 ml of this emulsion and 50 ml of bovine serum was incubated at room temperature for 30 min. The reaction mixture for determining activity consisted of 3 ml of substrate, 2 ml of 0.2 M Tris-HCl buffer, pH 7.6, and 1 ml of enzyme solution. The mixture was incubated at 30°C for 15 min with the determination of its optical activity at 660 nm before and after incubation. As the unit we took the amount of enzyme reducing the optical activity of the reaction mixture at 660 nm by 0.2 units in 15 min. The specific activity is the number of units of enzyme per 1 mg of protein.

Isoelectric focusing was carried out in an apparatus for the isoelectric focusing of proteins (Sweden) for 24 and 48 h at a temperature of 4°C, a current strength of 1.5 mA, and a final voltage of 800 V, in a linear 0-40% concentration gradient of sucrose with a 1% concentration of ampholines. Ampholines with a pH range of 3-10 were used. After the completion of the experiment, the contents of the column were eluted at a rate of 80 ml/h. The fractions were collected in an OE-603/1 automatic collector (Hungary). The optical densities of the protein were measured in an SF-16 spectrophotometer at 280 nm. The enzymatic activities of the eluates were analyzed after elimination of the sucrose and ampholines by gel filtration through Sephadex G-50, medium (Sweden).

Protein was determined by Lowry's method, and also by a spectrophotometric method with measurement of the optical densities at 260 and 280 nm.

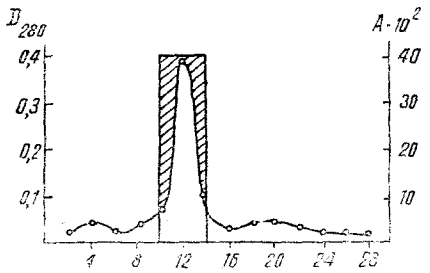

Fig. 3. Gel chromatography of the active fraction of CM-cellulose on Sephadex G-100 (column dimensions 1.2 × 85 cm, rate of elution 10 ml/h).

The electrophoretic investigation of the enzyme was performed by Davis' method [3] on a Reanal instrument (Hungary) in 7% polyacrylamide gel at pH 8.9.

Gel chromatography on Sephadexes G-50 and G-100, and also on DEAE-Sephadex A-50 was carried out according to the standard method [4].

Ion-exchange chromatography on CM-cellulose was performed in a Reanal product (Hungary).

The resin was treated by a known method [5].

SUMMARY

It has been shown for the first time that the fungus *Rhizopus microsporus* produces a lipoprotein lipase. A scheme for obtaining the homogeneous enzyme and some of its properties are described.

LITERATURE CITED

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