ISOLATION OF TRIACETINASE FROM THE SEEDS OF THE COTTON PLANT

M. M. Rakhimov, N. R. Dzhanbaeva, and P. Kh. Yuldashev

UDC 577.153+582.796

Continuing a study of the lipolytic enzymes of the seeds of the cotton plant [1, 2], we have isolated from them three enzymes of the triacetinase group (A, B, and C).

Triacetinase A was obtained from 330 g of an acetone powder isolated from dormant cotton seeds [1]. An extract in 0.1 M phosphate buffer (three liters) with pH 7.4 (2000 rpm, 30 min) was acidified to pH 3.8 with concentrated hydrochloric acid with vigorous stirring, the precipitate was separated off by centrifuging (6000 rpm, 30 min), and the supernatant was dialyzed against 10 volumes of 0.01 M phosphate buffer with pH 7.4. Subsequent chromatography on columns of DEAE-cellulose (4.5 × 100 cm and 3.0 × 55 cm) and columns of Sephadex G-50 (3.0 × 65 cm and 2.0 × 120 cm) gave an enzyme with a specific activity of 70,000 units/mg of protein (after each column stage of purification, the material was dialyzed and concentrated). The yield of purified enzyme was 30 mg. The purification factor was 14,000.

Triacetinase B was isolated from 20 g of the acetone powder. To an extract in 0.1 M phosphate buffer with pH 7.4 (18,000 rpm, 30 min) was added ammonium sulfate to 55% saturation at 4°C, and the precipitate was dissolved in the same buffer (20 ml) and was chromatographed on a column of Sephadex G-100 (3.4 × 70 cm). The fractions corresponding to a molecular weight of about 36,000 and possessing triacetinase activity (the column was previously calibrated with proteins of known molecular weights) were combined, freeze-dried, dissolved in 5 ml of double-distilled water, and dialyzed against 0.01 M phosphate buffer, pH 7.4. Then it was subjected to ion-exchange chromatography on DEAE-cellulose (column 1.0 × 22 cm), using for the elution of the protein a concentration gradient of sodium chloride up to 1 M. The yield of enzyme was 3.5-4.0 mg. Its specific activity was 50,000 units/mg of protein. The limiting purification factor of the protein achieved by this isolation procedure was 1050.

Triacetinase C was obtained similarly with the only difference that the fractions containing triacetinase and corresponding to a molecular weight of about 72,000 on gel filtration in a column of Sephadex G-100 (3.4 × 70 cm), before ion-exchange chromatography on DEAE-cellulose (1.0 × 22 cm), were rechromatographed on a column of Sephadex G-100 (1.0 × 22 cm). Yield 2.4 mg. Specific activity 21,000 units/mg of protein. Purification factor 525.

The homogeneity of the enzymes obtained was checked by rechromatography on molecular sieves (Sephadexes G-50 and G-100) and on an ion-exchange resin (with DEAE-cellulose, all three enzymes were eluted in gradient elution at a concentration of NaCl of 0.8 M) and also by ultracentrifugation. The triacetinase A was homogeneous on disk electrophoresis. The electrophoretic properties of triacetinases B and C will be discussed in connection with their quaternary structures.

The molecular weights of the homogeneous enzymes were determined by the following methods: 1) column gel filtration on Sephadex G-100 (18,000 for A, 36,000 for B, and 72,000 for C); 2) thin-layer gel filtration on Sephadex G-200 (18,000 for A, 38,000 for B, and 74,000 for C); 3) ultracentrifugation (19,200 for A and 35,600 for B); and 4) from the amino-acid compositions (18,500 for A and 34,800 for C).

The pH-optimum of the catalytic action of the enzymes was 9.5. The maximum activity was found at 40°C. The enzymes are capable of bringing about the hydrolysis of short-chain triglycerides (specific

---

Order of the Red Banner of Labor Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 119-120, January-February, 1974. Original article submitted May 4, 1973.

hydrolysis) and they also catalyze the cleavage of the ester bond in oils and in long-chain synthetic triglycerides.

LITERATURE CITED

1. M. M. Rakhimov, Sh. R. Mad'yarov, N. R. Dzhanbaeva, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 642 (1970).
2. N. R. Dzhanbaeva, M. M. Rakhimov, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 222 (1972).