THE SUBUNIT STRUCTURE OF A LIPASE INHIBITOR

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We have previously described a method for obtaining a lipase inhibitor from the fungus *Rhizopus microsporus* in the homogeneous state. The molecular mass was determined as 24,000-25,000 [1]. In the present paper we consider the results of study of the quaternary structure of the homogeneous lipase inhibitor.

The number of subunits in the lipase inhibitor was determined by the method of dissociation in solution of urea and of sodium dodecyl sulfate. A solution of the inhibitor was diluted with 8 M urea until its final concentration was 6 M, and gel filtration was carried out on a column of Sephadex G-75. As can be seen from Fig. 1, although both the inhibitor and the product of its dissociation were each eluted as a single symmetrical peak, they differed in elution volumes. It was established that the product of the dissociation of the inhibitor had a molecular mass of 12,000, which is half the molecular mass of the native inhibitor. The results of these investigations give grounds for stating that in the presence of 6 M urea the lipase inhibitor dissociates into two subunits. The monomer obtained in this way possesses no inhibiting action (Table 1).

TABLE 1. Influence of the Subunit and the Native Protein Inhibitor on the Lipase Activity of the Fungus *Rhizopus microsporus*

Concentration of subunits or native inhibitor, mg	Lipase activity, units		
	C	A_1	A_2
0,1	25 000	25 000	24 000
0,2	25 000	24 950	23 250
0,3	24 900	25 000	22 700
0,5	24 950	24 900	21 450
1,0	25 050	25 000	17 950

*C — control, units/mg; A_1 — fractions treated with the subunits of the inhibitor; A_2 — the same treated with the native inhibitor.

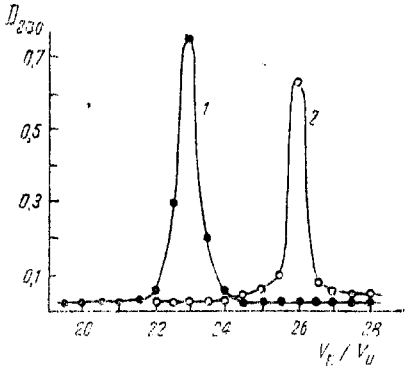

Fig. 1. Gel filtration of the lipase inhibitor and its subunits on a column of Sephadex G-200 (column dimensions 2 × 100 cm): 1) elution of the native inhibitor with 0.005 M phosphate buffer, pH 7.4; 2) elution of the dissociated inhibitor with the starting buffer containing 6 M urea and 7% of 2-mercaptoethanol.

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The elimination of urea by dialysis or filtration through a column of Sephadex G-25 did not lead to the reassociation of the subunits and the restoration of activity. On the basis of these experimental materials, it may be concluded that the dissociation of the lipase inhibitor in the presence of high concentrations of urea is irreversible.

The dissociating capacity of sodium dodecyl sulfate with respect to many proteins has been convincingly demonstrated. The lipase inhibitor was treated with sodium dodecyl sulfate in the presence of 2-mercaptoethanol. The products formed under these conditions were separated by disk electrophoresis in polyacrylamide gel containing sodium dodecyl sulfate. Under these conditions, the protein treated with sodium dodecyl sulfate had a relative electrophoretic mobility greater than that of the native inhibitor. The molecular mass of the dissociation product of the lipase inhibitor was calculated as $\approx 12{,}000$.

Thus, the lipase inhibitor can be assigned to the subunit protein consisting of two subunits the monomer of which possesses no inhibiting effect.

LITERATURE CITED

1. K. Davranov, Z. R. Akhmedova, and A. M. Bezborodov, Khim. Prir. Soedin., 373 (1983).