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PREPARATION OF THE [Ala^{16}-B] ANALOG OF BOVINE INSULIN

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In a study of the structural and functional organization of the insulin molecule we have obtained the previously unknown [Ala^{16}-B] analog (I) of bovine insulin.

The preparation of compound (I) was based on combining the natural A-chain of bovine insulin with the synthetic [Ala^{16}]-B chain of bovine insulin [1]. The protected synthetic chain was first demasked by treatment with sodium in liquid ammonia in the presence of sodium amide [2] and was then subjected to oxidative sulfitolysis [3]. This gave the bis-S-sulfonate (II) of the analog of the B-chain, in which the L-tyrosine residue in position B^{16} had been replaced by a L-alanine residue. The amino acid analysis of the bis-S-sulfonate (II) was: Phe 2.8, Val 3.0, Asp 1.0, Glu 3,0, His 2.0, Leu 4.0, Gly 3.0, Ser 0.7, Ala 3.1, Tyr 0.9, Arg. 1.0, Thr 0.8, Pro 0.9, Lys 1.0.

The bis-S-sulfonate (II) was brought into combination with the tetramercapto form of the A-chain of bovine insulin [4] previously prepared by treating the tetra-S-sulfonate of the A-chain of bovine insulin with 2-mercaptoethanol.

The compound (I) obtained was purified by ion-exchange chromatography on a column of CM-Sephadex C-25, using as eluent a 0.04 M acetate buffer solution with pH 4.0, and also by disk electrophoresis in polyacrylamide gel [5]. The purified compound (I) was isolated in the form of hydrochloride.

Compound (I) was homogeneous on paper electrophoresis ("Khromatograficheskaya M" paper, pH 2.6, 720 V, 10 mA) and in its electrophoretic mobility it did not differ from authentic natural bovine insulin. The homogeneity of the compound (I) was also confirmed on its disk electrophoresis in polyacrylamide gel (pH 8.3, concentration of separating gel 7.5%).

Amino acid analysis of compound (I): Gly 4.4, Ile 1.0, Val 4.7, Glu 7.3, Ala 4.0, Ser 3.0, Leu 6.0, Tyr 3.1, Asp 3.0, Phe 2.8, His 1.8, Arg 1.1, Thr 1.0, Pro 0.9, Lys 1.0.

The biological activity of compound (I) on testing for its convulsive effect in mice [6] was 50% (as compared with the activity of the international standard for insulin).

The results obtained show that the replacement in the molecule of bovine insulin of the invariant L-tyrosine residue in the B^{16} position by a L-alanine residue does not lead to the complete disappearance of the specific hormonal activity in the [Ala^{16}-B] analog of bovine insulin.

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ISOLATION AND PURIFICATION OF A LIPASE FROM THE FUNGUS *Oospora lactis*

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Under certain conditions, the fungus *Oospora lactis* produces an active exolipase (E.C. 3.1,1.3 — triglyceride hydrolase) [1]. In the present paper we discuss the isolation and purification of the extracellular lipase of the fungus *Oospora lactis* and some of its properties.

The mycelium of the fungus was separated by filtration through a paper filter. The enzyme was precipitated from the filtrate of the culture liquid with six volumes of isopropanol [1]. The enzyme was extracted from the "isopropyl powder" with 0.1 M phosphate buffer, pH 7.4. The part that did not dissolve was separated by centrifuging or by filtration through the paper filter. The clear solution of the enzyme was deposited on a column of Sephadex G-75 equilibrated with 0.1 M phosphate buffer. The dimensions of the column were 120 × 3 cm and the rate of elution 20 ml/h. Fractions with a volume of 5 ml were collected.

It can be seen from the elution graph (Fig. 1) that the protein issued in two peaks, and the lipase activity appeared between the two peaks (fractions 12-17). The active fractions were combined and were concentrated by freeze-drying or, in some cases, with the aid of dry, washed, Molselekt G-25 (Reanal) and redeposition on a column of Sephadex G-75 (2 × 100 cm). It was eluted with the initial buffer at the rate of 15 ml/h.

Activity was shown in the fractions of the second peak (Fig. 2). The specific acitivity of the enzyme had increase 100-fold in comparison with the isopropyl powder. A further attempt to purify the enzyme with the aid of DEAE-Sephadex A-50 gave no effect, the enzyme being eluted in one symmetrical peak. Disk electrophoresis in polyacrylamide gel [2] showed the presence of one band, which also gave the specific reaction for lipolytic enzymes [3].

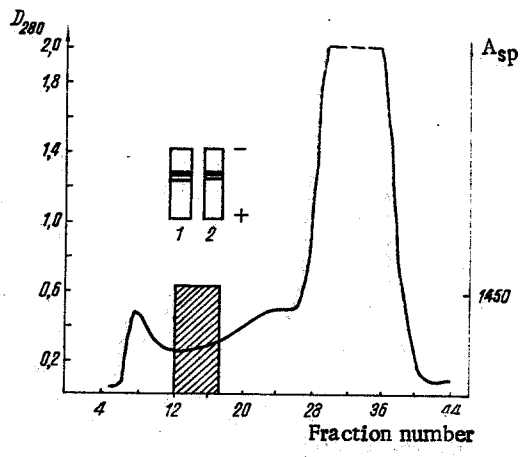

Fig. 1

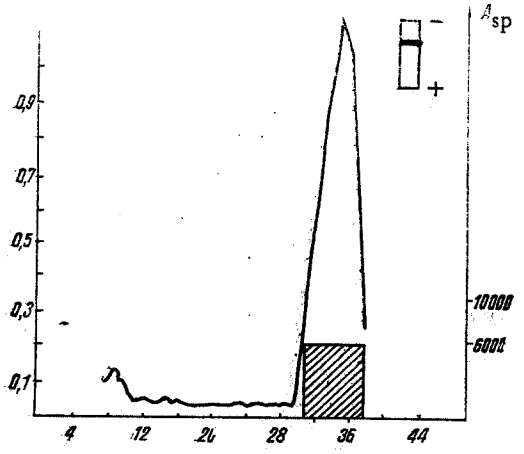

Fig. 2

Fig. 1. Separation of the total fraction on Sephadex G-75 (*Oospora lactis*): 1) electrophoretogram of the active fraction in polyacrylamide gel; 2) enzymogram (according to Abe) [3].

Fig. 2. Repeated gel filtration on Sephadex G-75 (*Oospora lactis*). For explanations, see Fig. 1.

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