PRIMARY STRUCTURE OF TRIACETINASE

III. AMINO-ACID SEQUENCE OF THE CYANOGEN BROMIDE FRAGMENTS

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We have previously reported the isolation and characteristics of the peptides of a cyanogen bromide hydrolyzate [1]. In the present communication we give the results of a study of the amino-acid sequences of the cyanogen bromide fragments.

The N-terminal amino-acid sequence was studied both by direct Edman degradation with the determination of the amino acids split off in the form of the Pth derivatives [2] and also by the Edman method in combination with dansylation [3]. The results of our experiments are given below:

Peptide	Amino-acid sequence
B-1	Arg-Gly-Asp-Gly-Asn-Ser-Glu-Ala-Homoser
B-2	Thr-Lys-Gln-Ala-Cm-Cys-Asp-Gln-Ser-Phe-Ala-Pro
B-3	Asp-Val-Ala-Asn-Gly-Gln-Gly-Thr-Gln-Lys-Arg-Ala-Gly-...
B-4	Pro-Trp-Asp-Thr-Arg-Ser-Ala-Gly-Asn-X-Pro-...
B-5	His-Ser-Asp-Gly-Cm-Cys-Thr-Ile-Arg-Thr-Arg-...
B-6	Arg-Gly-Asp-Gly-Asn-...
B-7	Asp-Val-Ala-Asx-Gly-...

The N-terminal amino-acid sequence of CM-triacetinase determined by the Edman method in the DNS modification was as follows: Met-Arg-Gly-Asx-Gly-Asx-Ser-... This sequence coincides with the N-terminal sequence of peptide B-1.

A comparison of information on the amino-acid composition [1] and N-terminal sequence of peptides B-1 and B-2 gives grounds for assuming that peptide B-6 is the product of the incomplete hydrolysis of peptides B-1 and B-2 at a methionine–threonine bond, which, as is well known, is resistant to the action of cyanogen bromide. Thus, it may be concluded that in the native triacetinase molecule peptide B-2 follows peptide B-1.

Similar conclusions can be drawn in a comparison of the amino-acid compositions [1] and sequences of peptides B-3 and B-4. Apparently, just as in the preceding case, peptide B-7 is a product of the incomplete hydrolysis of peptides B-3 and B-4 at a methionine–proline bond, which is also resistant to the action of cyanogen bromide [4] and, consequently, peptide B-4 follows peptide B-3.

Peptide B-5, containing tyrosine at the C-terminus, just like the native triacetinase, is obviously the C-terminal fragment of triacetinase.

So far as concerns peptide B-3, on analyzing the structure of B-2 (C-terminal amino acid proline) and taking into account the fact that the N-terminal amino acid is aspartic acid, it may be concluded unambiguously that fragment B-2 is a product of nonspecific hydrolysis (under the action of acid) of a proline–aspartic acid bond. Such cases have been described in the literature [5].

Thus, the positions of the cyanogen bromide fragments have been determined on the basis of information on the amino-acid compositions [1] and the N- and C-terminal sequences of the peptides of the cyanogen bromide hydrolyzate.

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ISOLATION OF A CYTOPLASMATIC REGULATOR MEDIATING THE ACTION OF HORMONES ON MITOCHRONDRIA

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The activating action of small amounts of liver cytoplasm (the supernatant from the centrifugation of a rat liver homogenate at 30,000g) on the transport of calcium in mitochondria (increase in the calcium capacity and rate of active transport) is regulated by the administration of insulin or epinephrine to rats. The administration of insulin (1 unit/200 g body weight) to rats 45 minutes before their sacrifice almost doubles the activity of the cytoplasm. The administration of epinephrine (1 mg/kg body weight) 15 min before sacrifice causes the opposite effect. This is in harmony with the antagonism in the action of insulin and epinephrine on the liver metabolism.

The cytoplasmatic factor the activity of which is regulated by the level of the hormones was isolated and purified by successive heating at 95°C for 7 min, gel filtration through Sephadex G-25 (column equilibrated and eluted with 0.1 M KCl), chromatography on DEAE-cellulose, and electrophoresis in polyacrylamide gel. According to the results of gel filtration, the molecular weight of the regulator is about 5000 daltons. It was eluted from the DEAE-cellulose column with 0.35 M KCl. Before electrophoresis, the regulator was desalted on a column of Sephadex G-10. The hormone-dependent regulator from the cytoplasm acts on the mitochondria in very low concentrations, of the order of 10^{-8}. In many of its parameters, it differs from the nucleotide factor which we isolated previously from rabbit liver cytoplasm [1].

There are no nucleotides in purified preparations of the hormone-dependent regulator.

An intense band in the IR spectrum with a maximum at 1045 cm^{-1} may apparently be related to the skeletal vibrations of a P–O–C group, as is confirmed by elementary analysis for phosphorus. The presence of organic phosphorus in the molecule explains its high affinity for DEAE-cellulose. The presence of peptide bonds in the preparation was shown by the biuret method, and is also confirmed by the presence of characteristic bands in the IR spectra (amide I band with its maximum at 1670 cm^{-1}, and amide II band with its maximum at 1600 cm^{-1}). A considerable amount of sugar was found in a hydrolyzate of the preparation by the "Dubois" method, which shows the glycoprotein nature of the hormone-dependent regulator.

An investigation of the amino-acid composition of the glycoprotein showed the presence in it of a polypeptide consisting of 42 amino acids: aspartic acid 3; threonine 1; serine 7; glutamic acid 6; proline 4; glycine 6; alanine 3; valine 1; isoleucine 1; leucine 1; tyrosine 1; phenyl alanine 1; histidine 1; lysine 5; arginine 1.

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