THE ISOLATION OF IODINE-CONTAINING PEPTIDES OF THYROGLOBULIN

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Thyroglobulin was isolated from an extract of the tissue of bovine thyroid gland by two gel filtrations on Sephadex G-200 [1]. The protein was reduced in 8 M urea at pH 10.3. For the complete cleavage of disulfide bridges, β-mercaptoethanol was used in a molar ratio to semicystine of 20:1 [2]. The reaction was performed at 25°C for 45 min. Alkylation was performed with iodoacetic acid at a molar ratio to β-mercaptoethanol of 1.5: 1, pH 10.3. The time of incubation was 2.5 h at 37°C. The pH of the reaction mixture was maintained by the addition of 0.1 M KOH. The reduced carboxymethylated protein (RCP) was concentrated in a rotary evaporator to a concentration of 10 mg/ml. It was hydrolyzed with trypsin (Serva) in a ratio to the substrate of 1:25 at 37°C for 72 h; pH 8.2, which was kept constant by the addition of 0.1 M KOH. The hydrolyzate was separated by descending paper chromatography in the butanol—acetic acid—water (4:1:5) system. The chromatograms were stained with ninhydrin. The iodine-containing fractions were identified by Postmes's method [3]. To determine the nature of the iodine-containing components of the iodine-containing fractions, the latter were subjected to alkaline hydrolysis with $Ba(OH)_2$ followed by paper chromatography in the butanol—ethanol—ammonia (5:1:2) system. To identify the iodoamino acids we used standard solutions of T_3, T_4, DIT, and MIT. The amount of N-terminal amino acids was determined by the Dansyl-Edman method [4], and of SBI by Shtol'ts's method [5].

Hydrolysis yielded hydrophobic and hydrophilic peptides which were separated by centrifuging. The results of a determination of SBI in these fractions of peptides showed that 75% of the iodine of the protein was present in the hydrophilic peptides formed on the hydrolysis of thyroglobulin.

Chromatography of the hydrophilic fractions showed 20 clearly separated spots [stained with ninhydrin). Staining by Postmes's method showed that four of them contained the bulk of the iodine. These spots (peptides) have been arbitrarily denoted P1, P2, P3, and P4. The results of a determination of SBI in these four fractions showed that 0.3% of the total amount of iodine in all the hydrophilic peptides was present in the P1 fraction, 42% in P2, 53% in P3, and 1.7% in P4. In an analysis of the nature of their iodine-containing components it was found that fractions P2 and P3 contained inorganic iodine, DIT, and T_4, fraction P1 contained DIT and inorganic iodine, and P4 contained DIT.

To determine the individualities of the fractions obtained we determined their N-terminal amino acids. In the peptides of each spot we found several N-terminal amino acids. Since we are interested in the T_4-containing sections of thyroglobulin, fractions P2 and P3 will be subjected to further treatment and purification.

LITERATURE CITED

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