STUDY OF THE IODINE-CONTAINING PEPTIDES OF HUMAN THYROGLOBULIN ON THE BASIS OF THE RESULTS OF A NEUTRON-ACTIVATION ANALYSIS

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We have determined the amino-acid composition of the iodine-containing peptides of thyroglobulin obtained from goitrously changed human thyroid glands. In this glycoprotein, 10% of the molecular weight of which is due to carbohydrate components (351 moles of carbohydrates per mole of protein with a molecular weight of 660,000), 202 sulfhydryl groups have been found [1, 2].

The protein was obtained by Karlsson's method [3] in our modification [4]. Enzymatic hydrolysis of the thyroglobulin (without preliminary cleavage of the disulfide bonds) was performed with pronase (a Japanese product tested in the protein chemistry laboratory of the Institute of Organic Chemistry of the Academy of Sciences of the USSR) at 38-39°C in a volatile buffer [0.1 M $(CH_3CH_2)_3N$ and 0.1 M CH_3COOH], pH 8.0, for 5 h. The amount of protein used for each hydrolysis was 1.8-2.2. The small precipitate was separated by centrifuging, and the hydrolyzate was subjected to two-dimensional paper chromatography in systems 1) water-saturated phenol and 2) butan-1-ol – acetic acid–water (4:1:5), as a result of which 15 peptides were obtained. The neutron-activation analysis of these peptides showed that the iodine was concentrated mainly in five of them: the second, the 12th, the 13th, the 14th, and the 15th.

The peptides richest in iodine (the 14th and 15th) were investigated after their homogeneity has been checked by paper electrophoresis in various buffers (CH_3COOH and CH_3COONa; KH_2PO_4 and Na_2HPO_4; veronal and medinal) at various pH values (4.7, 6.8, and 8.6). The hydrochloric acid hydrolysis of the 15th peptide (5.7 N HCl, 24 h, boiling water bath) formed a dark-brown human-like oily precipitate. This could be the result of the far-reaching cleavage of carbohydrates (sialic acids) and, possibly, tryptophan, only about 83 moles of which are present in 1 mole of the protein (mol. wt. 660,000) [5]. The carbohydrates were detected by the Molisch reaction. When the 15th peptide was subjected to baryta hydrolysis [saturated solution of $Ba(OH)_2$, sealed capillary, 13 h, boiling water bath], a sharp smell of ammonia rose which could be the consequence of the decomposition of amino-acid amides, and also of amino sugars formed in the decomposition of the resinous substance [6].

In a determination of the amino-acid composition of the iodine-containing peptides of thyroglobulin it was found that all the peptides apart from the 13th contained cystine, the presence of which was confirmed by the iodine-azide reaction [7]; the 14th peptide contained basic amino acids (lysine, arginine, and histidine). Of the monoamino dicarboxylic acids, glutamic acid was present in all the peptides, and aspartic acid in the 2nd, 13th, and 15th. The 15th peptide also contained cystine, cysteinic acid, and those amino acids through which carbohydrate units can be attached to a peptide chain (serine, glutamic and aspartic acids, threonine).

Thyroxine was present in two peptides – the 12th and the 15th. A comparison of these peptides showed the very great similarity of their amino-acid compositions. Nine amino acids are common to both peptides: cystine, serine, glutamic acid, threonine, tyrosine, valine, glysine, leucine, and thyroxine. The 12th peptide contained, in addition, lysine, alanine, and diiodotyrosine, while in the 15th peptide, in addition to the nine common amino acids, there were aspartic acid and phenylalanine.

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The similarity of the amino-acid compositions of the 12th and 15th peptides permitted the assumption that the amino-acid environment of thyroxine in the protein plays a decisive role, i.e., it is possible that in the native protein the amino acids present close to the section of the protein where thyroxine is synthesized (in vivo) ensure the appropriate conformation of these sections of the thyroglobulin molecule and, apparently, thyroxine is synthesized (in vivo) under completely definite conditions in which a not unimportant role is played by the surrounding amino acids.

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