AN INVESTIGATION OF THE THYROXINE-CONTAINING PEPTIDE OF HUMAN THYROGLOBULIN

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Thyroglobulin (TG) is an iodine-containing protein of the thyroid gland the specific nature of which is due to the presence of iodinated thyronines in it. The molecular weight of TG is $6.5 \cdot 10^6$ [1], and its sedimentation coefficient is 19S. This protein contains two types of chains [2-5].

We obtained the iodine-containing protein TG by Karlsson's method with our own modifications [4]. Without preliminary reduction of the disulfide bonds, the protein was hydrolyzed with pronase at 40°C, pH 8.0, for 5 h. After two-dimensional chromatography in aqueous phenol and in the butan-1-ol – acetic acid – water (4:1:5) system on paper freed from Na^+ and Cl^- ions [6], about 15 peptides were detected, and in these the distribution of iodine was determined by neutron-activation analysis [6]. For this purpose, sections corresponding to the peptides found were cut from the chromatograms and were irradiated for 5 min in the channel of a reactor with a flux of 10^{13} neutrons/cm^2 · sec. The induced activity was measured in an apparatus consisting of a USD-1 scintillation counter and an AI-100 one-hundred-channel analyzer. The activity of the sample was calculated by means of the formula for activation analysis [6]. The peptides richest in iodine were eluted from the chromatogram with distilled water, dried, and hydrolyzed with 6 N HCl for 24 h in sealed capillaries in the boiling water bath.

Paper chromatography of the hydrolyzates showed that thyroxine was present in the 15th peptide. (The numbering of the peptides is arbitrary.) In addition to thyroxine, the 15th peptide contained cystine, serine, glycine, leucine, threonine, aspartic and glutamic acids, tyrosine, phenylalanine, valine, and diiodotyrosine. Electrophoresis of the 15th peptide on paper, performed in various buffers at various pH values (4.7, 6.8, and 8.6), showed that the substance was homogeneous.

To determine the sequence of amino acids in the thyroxine-containing peptide, the peptide was eluted [7] from paper chromatograms of a hydrolyzate of the protein stained with a dilute solution (0.015%) of ninhydrin. Analysis of the N-terminal amino acids and subsequent degradations were performed by a method which we have described previously [4].

The results obtained show that thyroxine and glycine are located at the N end of this peptide. Subsequent degradations gave glycine and tyrosine, serine and tyrosine, and also glutamic acid and threonine.

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