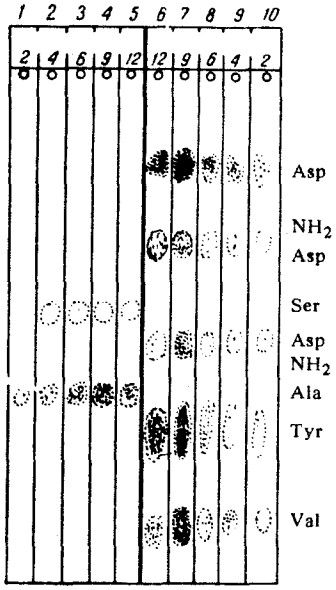
For hydrolysis with hydrochloric acid, the PTHs must be subjected to prolonged heating at 150° C, which leads to considerable losses of the regenerated amino acids. Hydrolysis with baryta has the same disadvantages, and the necessity for eliminating Ba^{++} after hydrolysis increases the losses of amino acids due to their adsorption on the massive precipitates of $BaSO_4$ or $BaCO_3$, which complicates the process. Consequently, we used ammonia hydrolysis on a boiling water bath. This method enables the hydrolysis to be carried out more rapidly and at a moderate temperature; it is particularly suitable for the identification of tryptophan.

The optimum hydrolysis period, giving the greatest amount of free amino acids, proved to be 9 hours. The possibility of using the method was checked in a determination of the N-terminal amino acids in the peptides of silk fibroin and in mung bean globulin. An ethanolic or ethyl acetate extract of the PTHs was evaporated in vacuum [5] and the residue was repeatedly extracted with cord ammonia and hydrolyzed in a sealed capillary. Completely satisfactory results were obtained (figure).

The new method of the hydrolysis of the PTH-amino acids with ammonia is proposed for the direct identification of the N-terminal amino acids. In this method, serine is almost completely decomposed and aspartic acid is partially converted into the α- and γ-amides.



Chromatogram of the amino acids liberated from the phenylthiohydantoins. 1–5) Hydrolyzates of the PTH derivatives of alanine and serine, 6–10) hydrolyzates of the PTH derivatives of valine, tyrosine, aspartic acid, and asparagine after 2, 4, 6, 9, and 12 hr.

REFERENCES

1. J. Sjöquist, J. Biochem. Biophys. Acta, 41, 20, 1960, and earlier.
2. P. Edman, Acta Chem. Scand., 4, 277, 283, 1950.
3. H. Fraenkel-Konrat, J. I. Harris, and A. L. Levy, In D. Glick (ed.), Methods of Biochemical Analysis, **2**, 383, 1955.
4. D. G. Elliot and W. S. Peart, Biochem. J., 65, 246, 1957.
5. H. O. Van Orden and Frederick Carpenter, Biochem. and Biophys. Res. communic, **14**, 5, 399–403, 1964.

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N-TERMINAL AMINO ACIDS OF HUMAN THYROGLOBULIN

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Thyroglobulin (TG) is a physiologically active protein of the thyroid gland. Its molecular weight is 6.5×10^6 [1] and its sedimentation coefficient 19. In a determination of the N-terminal amino acids of the protein [3–5], nine different amino acids were found, which suggests the presence of nine peptide chains in it.

We have determined the N-terminal amino acids in TG and have therefore found the true number of polypeptide chains.

We obtained thyroglobulin from human nontoxic goitrous thyroids [6] by Karlsson's method [7] with our modification of ultrafiltration through 1–3% perlodium. The lyophilized protein was electrophoretically homogeneous at pH 4.7–6.7 and 8.5. A sedimentation analysis showed that 90% of the protein had 19S, 5% had 27S, and 5% had 12S. The appearance of 27S and 12S is explained by the influence of the preliminary lyophilization of the protein. The protein contained 15.5% of nitrogen.

In order to avoid steric hindrance [5], the sialic acids were removed by Spiro's method [2]: 50 mg of a mixture of the sample and salt (about 5 mg of pure protein) was dissolved in 0.3 ml of water. The solution was treated with 1 N sulfuric acid to pH 1.5 and then 0.05 N sulfuric acid was added to give a 0.3% solution of the protein and this was heated in the water bath at 80° C for 1 hr. The protein was freed from sialic acid by dialysis (initially against 0.1 M NaCl and then against water), and the protein precipitate was collected in the centrifuge.

Edman degradation in Eriksson and Sjöquist's modification [8] was carried out with some of our own modifications. To the protein precipitate was added 1 ml of water and 2 ml of a mixture of pyridine, triethylamine, and phenyl isothiocyanate (100:3:1). The mixture was thermostated at 40° C for 1.5 hr, the excess of solvent was removed by extraction with benzene 7–8 times, and the mixture was dried over phosphorus pentoxide or sodium hydroxide and paraffin wax. Cyclization was carried out by our combination of the two methods of Sjöquist [8] and Light [9]. To the PTH-protein were added 1 ml of water and then 2 ml of a mixture of glacial acetic acid and concentrated hydrochloric acid (5:1) and the resulting mixture was thermostated at 40° C for 2 hr, after which it was dried over sodium hydroxide in vacuum. The dry residue was treated with 2 ml of water saturated with a mixture of ethyl acetate and methyl ethyl ketone. The PTH-derivatives were extracted with a mixture of ethyl acetate and methyl ethyl ketone (2:1) and dried by Belitser's method [10].

Hydrolysis of the PTH-amino acids was carried out with 11.86 N ammonia for 14–16 hr [11]. Descending one-dimensional chromatograms were carried out in aqueous phenol and in the butanol—acetic acid—water (4:1:5) system. On each of the two chromatograms of the material studied we obtained two spots. On comparing the spots obtained with reference samples it was found that the N-terminal amino acids of human thyroglobulin are alanine and glycine. Consequently, the molecule of thyroglobulin consists of two chains beginning with alanine and glycine.

REFERENCES

1. B. de Crombrugghe, R. Pitt-Rivers, and H. Edelhoch, J. Biol. Chem. 241, 2766, 1966.
2. R. G. Spiro and M. J. Spiro, J. Biol. Chem., 240, 997, 1965.
3. J. Roche, R. Michel, and J. Nunez, Bull. Soc. Chem. Biol., 37, 219, 1955.
4. J. Roche, R. Michel, and J. Nunez, Bull. Soc. Chem. Biol., 37, 229, 1955.
5. T. A. A. Dopheide and V. M. Trikojus, Nature, 201, 1128, 1964.
6. I. G. Pierce, A. B. Rawitch, D. M. Brown, and P. G. Stanley, Biochim. Biophys. Acta, 111, 247, 1965.
7. R. Karlsson, V. Kivikangas, and B. A. Lamberg, Acta Chem. Scand., 17, 2347, 1963.
8. S. Eriksson and J. Sjöquist, J. Biochim. Biophys. Acta, 45, 290, 1960.
9. A. Light and J. Greenberg, J. Biol. Chem., 240, 258, 1965.
10. V. A. Belitser and N. N. Orlovskaya, Biokhimiya, 29, 741, 1964.
11. G. Ya. Lamm, A. R. Rakhimov, K. G. Ioffe, and I. A. Asatov, KhPS [Chemistry of Natural Compounds], 62, 1968.

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A STUDY OF THE WATER-SOLUBLE FRACTION OF THE PROTEINS OF THE SEEDS OF THE COTTON PLANT

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In the Laboratory of the Chemistry of Plant Proteins of the Institute of the Chemistry of Plant Substances, AS UzSSR, a study of the protein composition of the seeds of the cotton plant (variety 108-F) has been begun. The seeds freed from the pods and comminuted were defatted [1] (total nitrogen 8.72, protein nitrogen 7.65% of the weight of the absolutely dry defatted meal). To select the conditions for the maximum extraction of the water-soluble fraction of the proteins we used extraction with water at 0° C and extraction with 10% sodium chloride (total nitrogen in the extract 6.68; protein nitrogen 6.19%). The extracts obtained were dialyzed and the globulin components that deposited were separated off by