dipeptide, chromatographically homogeneous [R_f 0.42-0.43, system 1 (1:1)].

5. Preparation of Boc-Lys(Z)-Lys(Z)-OMe. By the method of paragraph 1, 5.0 g (17 mmol of H-Lys(Z)-OMe and 5.8 g (15.3 mmole) of Boc-Lys(Z)-OH gave, after recrystallization from diethyl ether, 7.5 g (75%) of the corresponding dipeptide, chromatographically homogeneous [R_f 0.40-0.46, system 1 (9:1)].

SUMMARY

1. A number of peptides have been obtained from L-lysine by the application of the silylation method.

2. The peptides obtained can be used as intermediates in the synthesis of biologically active substances.

LITERATURE CITED

1. M. V. Kashutina, S. L. Ioffe, and V. A. Tartakovskii, Usp. Khim., 44, 1620 (1975).
2. A. E. Pierce, Silylation of Organic Compounds, Pierce Chemical Co., Rockford (1968).
3. R. Piekos, Wiadom. Chem., 22, 543 (1968).
4. S. V. Rogozhin, A. I. Yurtanov, Yu. A. Davidovich, S. M. Andreev, and N. V. Mironov, Izv. Akad. Nauk SSSR, Ser. Khim., 1868 (1974).
5. J. F. Klebe, H. Finkbeiner, and D. M. White, J. Am. Chem. Soc., 88, 3390 (1966).

INFLUENCE OF ACETYLATION ON THE IODINATION OF THYROGLOBULIN AND THE FORMATION OF THYROXINE

T. A. Babaev, I. G. Akhmedzhanov,
E. E. Gussakovskii, and K. M. Ermolaev

UDC 577.15.049

The synthesis of thyroid hormones in the thyroglobulin molecule includes the condensation of iodotyrosine residues to form iodothyronines [1]. A fundamental role in this process is apparently played by the conformational state of the thyroglobulin [2, 3], since the steri propinquity of the iodotyrosine residues must favor their reaction with one another [1].

Since the charged groups of the protein molecule interact with one another [4], the replacement of such groups by neutral groups should lead to a change in the structure of the protein. The aim of the present work was to determine the influence of such a change on the capacity of thyroglobulin for hormone formation on additional iodination *in vitro*. The replacement of the NH_3^+ groups in thyroglobulin was performed by acetylating the arginine and (or) lysine residues.

2-Diacetylaminocyclohex-2-enone (DACH) is capable of acetylating the bases of α,ω-diamino acids (L-lysine, L-ornithine) with the predominant formation of N^{ω}-acetyl derivatives and conversion into ACH [5]. The capacity for DACH for acetylating the ω-NH_3^+ groups of lysine residues in porcine pepsinogen is also known [6]. In view of this, the acetylation of thryoglobulin was carried out with DACH.

The results of amino acid analyses of the intact and acetylated thyroglobulins are given in Table 1. To calculate the number of residues in the protein we used the molecular weight of 670,000 [1].

Intact thyroglobulin contains 270-280 arginine and 152-168 lysine residues. The acetylated thyroglobulin contained approximately 64 arginine residues less than the original substance. The number of lysine residues scarcely changed. No changes in the amounts of the other amino acids were observed, either.

Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Institute of Biological and Medicinal Chemistry, Academy of Medical Sciences of the USSR, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 485-488, July-August, 1978. Original article submitted March 1, 1978.

0009-3130/78/1404-0412$07.50

TABLE 1. Amino Acid Compositions of Intact and Acetylated Thyroglobulins

Amino acid	Amt. of amino acid, mg/100 mg protein		I–A
	acetylated (A)	intact (I)	
Asp	7,68±0,431	7,96±0,756	0,28±1,187
Thr	5,11±0,318	5,07±0,082	0,04±0,400
Ser	8,65±0,311	8,45±0,165	0,20±0,476
Glu	14,29±0,237	14,12±0,334	0,17±0,571
Pro	9,95±0,932	9,06±0,770	0,89±1,702
Gln	7,57±0,085	7,62±0,132	0,05±0,217
Ala	8,11±0,092	8,12±0,037	0,01±0,129
Val	4,63±0,850	5,00±0,200	0,37±1,050
Met	0,84±0,640	0,85±0,007	0,01±0,647
Ileu	2,37±0,092	2,30±0,037	0,07±0,129
Leu	8,98±0,951	8,70±0,470	0,28±1,421
Tyr	2,41±0,057	2,40±0,060	0,01±0,117
Phe	5,93±0,184	5,58±0,050	0,35±0,234
Lys	3,66±0,120	3,51±0,190	0,15±0,310
His	1,59±0,060	1,52±0,044	0,07±0,104
Arg	5,55±0,106	7,22±0,177	1,66±0,283
Cys	2,52±0,262	2,33±0,254	0,19±0,516

TABLE 2. Iodo Amino Acid Composition of Thyroglobulin (moles per mole of protein)

Iodo amino acid	Thyroglobulin			
	I	A	Io	A+Io
Tyrosine	109,38±0,77	106,86±0,77	93,32±0,89	100,45±2,29
Monoiodotyrosine	7,52±0,38	7,14±0,43	11,93±0,30	10,25±0,44
Diiodotyrosine	7,52±0,13	7,00±0,27	13,73±0,49	10,44±1,04
Thyroxine	3,31±0,40	4,75±0,48	5,81±0,62	5,16±0,54
Σ	130,63±0,04	130,47±1,14	130,32±0,93	131,09±0,68

Note. I) intact; A) acetylated with 1.5-2 moles of DACH per mole of (Lys + Arg); Io) iodinated with 200 moles of I_2 per mole of protein. The molecular weight of the thyroglobulin was taken as 6.7×10^5 [1]. Σ) Sum of the aromatic residues of tyrosine, monoiodotyrosine, diiodotyrosine, and thyroxine.

The results of spectrophotometric titration (Table 2) showed that the iodination of thyroglobulin *in vitro* forms new iodo amino acids with a simultaneous decrease in the number of tyrosine residues, the amount of diiodotyrosine increasing by approximately 6.2 residues, of monoiodotyrosine by 4.4 residues, and of thyroxine by 2.5 residues.

The acetylation of thyroglobulin without subsequent iodination led to an increase in the amount of thyroxine by 1-1.5 residues. However, the amount of newly synthesized thyroxine residues in the acetylated protein after its iodination scarcely differed from the number of newly formed thyroxine residues in the iodination of the intact thyroglobulin. At the same time, the acetylation of the protein suppressed the increase in the amount of MIT and DIT at the same level of iodination of the acetylated and the intact thyroglobulins by 1.7 and 3.3 residues, respectively.

In thyroglobulin, DACH selectively acetylates about 24% of the arginine residues at an acetylation level of 1.5-2 moles of DACH per mole of protein. At the same time, the lysine residues are not acetylated. Apparently, the arrangement of the lysines in the thyroglobulin globule prevents the modification of their ω-amino groups, since DACH is capable of acetylating lysine both in solution and as part of a protein [5, 6]. At the same time, it is not excluded that acid hydrolysis leads to partial deacetylation.

The increase in the amount of iodo amino acid residues in the intact thyroglobulin on its iodination corresponds to earlier results [1, 9]. The iodination of acetylated thyroglobulin also leads to the formation of new MIT, DIT, and T_4 residues, but their total number and their quantitative ratio change in comparison with the result for the intact protein at the same level of iodination. It is possible that when the ω-amino groups of the arginine

residues are blocked, a transformation of the structure of thyroglobulin takes place in such a way that the number of tyrosines capable of iodination in the intact protein decreases.

At the same time, such a transformation of the thyroglobulin structure does not affect the formation of new molecules of thyroxine on iodination *in vitro*. This possibly means that the formation of thyroxine in thyroglobulin on iodination *in vitro* takes place, at least partially, from the iodotyrosine residues present in the protein before iodination. Furthermore when the intact protein is acetylated, new molecules with a thyronine structure are formed in it. It is important that in these circumstances the number of iodotyrosine residues does not decrease, but the number of tyrosine residues falls.

The decrease in the number of tyrosine residues is double the increase in the number of thyroxine residues. However, in each molecule of thyroxine there are four iodine atoms that are absent from tyrosine. This contradiction can be explained in two ways: either there are adsorbed iodine atoms in thyroglobulin that are not bound to tyrosine residues, or the method of determining the thyroxine does not provide the possibility of distinguishing thyronine structures containing and not containing iodine.

A comparison of information on the determination of iodine in thyroglobulin by the chemical method and the reckoning of the number of iodine atoms from the amount of iodo amino acid residues give the same result, but with a large error (substantially more than four iodine atoms per protein molecule) [1]. On the other hand, the spectrophotometric method of determining thyroxine does not imply the separate determination of thyroxine and triiodothyronine simultaneously. Thus, the two possibilities are equally valid. Consequently, the answer to this question requires a special investigation.

In spite of the contradiction mentioned, the formation of a thyronine structure on the acetylation of the arginine residues of thyroglobulin remains an undoubted fact.

EXPERIMENTAL

The thyroglobulin was isolated from bovine thyroid gland by the fractionation of salt extracts of thyroid tissue on a column of Sephadex G-200 [7, 8]. The concentration of the thyroglobulin was determined spectrophotometrically ($A^{1\%}_{1cm}$ 11.5). The thyroglobulin was subjected to modification in 0.1 M phosphate buffer at pH 9.0 by the addition of DACH to a solution of the protein at the rate of 1.5-2 moles of DACH per mole of (Lys + Arg). Incubation was carried out at 35-37°C for 1 h. The acetylated thyroglobulin was subjected to gel filtration on a 1.5 × 30 cm column of Sephadex G-25 in order to free it from unchanged DACH and ACH.

Since the acetylation of lysine or arginine residues should lead to a decrease in their number in the protein, the process of acetylation was monitored by analyzing the amino acid composition of the thyroglobulin on a "Khron-1200E" amino acid analyzer. The protein was hydrolyzed with 6 N HCL at 105°C for 24 h. The evaporated hydrolyzate was dissolved in citrate buffer, pH 2.2.

The thyroglobulin was iodinated *in vitro* in 0.1 M phosphate buffer at pH 9.0 and 30°C as described elsewhere [9]. To 1 ml of a 1% solution of the intact or acetylated thyroglobulin was added the iodinating reagent (0.48 M KI + 0.04 M I_2) in an amount of 200 moles of I_2 per mole of protein, with constant stirring, and the mixture was incubated at 30°C for 30 min. The iodine that had not reacted with the protein was eliminated by dialysis against distilled water.

The numbers of tyrosine and iodo amino acid residues in all the samples of thyroglobulin were determined by spectrophotometric—pH-metric titration according to Edelhoch [9].

Assistance was given by Sh. Yunuskhanov in the determination of the amino acid composition.

SUMMARY

The results obtained show that when thyroglobulin is iodinated *in vitro* the formation in it of thyroxine from the iodotyrosine residues present in it before iodination is possible. The structure of the thyroglobulin apparently plays an important role not only in the formation of thyroxine but also in the level of iodination of the protein.

LITERATURE CITED

1. Ya. Kh. Turakulov, T. A. Babaev, and T. Saatov, Iodoproteins of the Thyroid Gland [in Russian], Tashkent (1974).
2. I. Nunes, I. Mauchamp, I. Pommier, T. Cirkovic, and I. Roche, Biochem. Biophys. Res. Commun., 23, 761 (1966).
3. E. E. Gussakovskii and T. A. Babaev, Abstracts of the Second All-Union Conference on the Spectroscopy of Biopolymers [in Russian], Khar'kov (1974), p. 40.
4. R. Lumry and R. Biltonen, in: Structure and Stability of Biological Macromolecules, (ed. S. N. Timasheff and G. Fasman), Marcel Dekker, New York (1969).
5. K. M. Ermolaev, N. A. Popova, and A. I. Tochilkin, Zh. Vses. Khim. O-va., 14, 357 (1969).
6. V. L. Shpitsberg, T. N. Shaposhnikova, and K. M. Ermolaev, Khim. Prirodn. Soedin., 129 (1971).
7. Y. Mouris and Y. B. Stanbury, Can. J. Biochem., 46, 51 (1968).
8. T. A. Babaev, A. A. Avanesova, E. E. Gussakovskii, and T. Usmanov, Biokhimiya, 37, 1306 (1972).
9. H. Edelhoch, J. Biol. Chem., 237, 2778 (1962).

ISOLATION OF THE CYTOPLASMATIC 5S RIBOSOMAL RNA FROM COTTON SEEDS BY PREPARATIVE ELECTROPHORESIS

A. M. Érmatov and R. N. Nuriddinov

UDC 547.963.3:633.51:537.363

The cytoplasmatic ribosomes of the seeds of the cotton plant of variety 108-F were isolated by a method described previously [1]. The total ribosomal RNA was obtained from the ribosome by sodium dodecyl sulfate (SDS)—phenol treatment. Analysis of the total rRNA by electrophoresis in 10% polyacrylamide gel (PAG) showed that the preparation contained 95-96% of high-molecular-weight ribosomal RNAs (28S + 18S), 1.2-1.5% of 5S rRNA, and 2.4-3% of tRNA, which agrees with literature information [2].

For the fractionation of RNA and the separation of the 5S rRNA from other components, some authors have used the method of gel filtration on Sephadex G-1000 [3] or G-75 [4], or on Sephadex G-100 followed by rechromatography on G-75 [5], chromatography on MAK (methylated albumin on kieselguhr) [6], and fractionation by electrophoresis in PAG [7, 8]. Electrophoresis in PAG possesses a higher resolving capacity than the other methods of fractionation and permits the 5S rRNA fraction to be separated in homogeneous form even when products of the degradation of high-molecular-weight rRNA are present in the preparation of total RNA.

Various instruments are used for preparative electrophoresis in polyacrylamide gel [9, 10]. Following the design of Maizel's instrument [11], with some modifications, we have put together an instrument for preparative gel electrophoresis with continuous elution of the substances being separated which permits the fractionation and separation of homogeneous fractions of 5S rRNA and of the total tRNA from the total rRNA.

To enrich the total rRNA with the 5S rRNA fraction and also to avoid the wrinkling of the surface of the gel and its detachment from the walls of the chamber caused by an excess of high-molecular-weight rRNAs, the latter were separated from the preparation by salting out with sodium chloride [12]. We used NaCl solutions of various concentrations (from 1 M to saturation) at concentrations of the sample of total rRNAs of 150-200 o.u./ml and at pH values of the solution of 5.6, 7, and 8.

At a concentration of total rRNA in the solution of 150 o.u./ml, the percentage of high-molecular-weight rRNAs in the preparation after salting out with 1 M NaCl solution was 56%, with 2 M solution 46%, with 3 M 39%, and with 4 M 38%. However, an increase in the concentration of total rRNA to 200 o.u./ml and of the concentration of NaCl to saturation (26 g per 100 g of solution) led to a decrease in the amount of the high-molecular-weight rRNA fraction in the preparation of total rRNA. Thus, after salting out with 4 M NaCl solution the amount

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 489-494, July-August, 1978. Original article submitted April 26, 1978.

0009-3130/78/1404-0415$07.50