PROTEIN COMPOSITION OF THE COLLOID COLLECTED FROM SINGLE RAT THYROID FOLLICLES

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Summary

By means of a micropuncture technique the luminal contents of single rat thyroid follicles were extracted. Analysis of the proteins was performed by micro-disc electrophoresis. The protein composition of the colloid varied between follicles of the same gland. In most follicles 19S thyroglobulin was the predominant protein and in addition 27S and 12S proteins were found in varying relative amounts. In some follicles a 4S protein resembling serum albumin with regard to electrophoretic mobility was observed in addition to the other proteins.

Key-words

Micropuncture - thyroid follicles - Micro-disc electrophoresis - Iodoproteins - Thyralbumin.

About thirty years ago Gersh and Baker (1) investigated the nature of the colloid in the thyroid follicles by U.V. absorbance analyses of sections of the gland. They stated that the follicle lumen probably contained thyroglobulin and that the protein concentration and iodination of the colloids vary with different functional states. Since then no further studies of the proteins in the colloids have been performed, apparently due to methodological limitations. The disc electrophoresis technique (2, 3), however, opened a new way for analysis of dilute samples of proteins and its micro-scale modification (4) proved to be sensitive enough for separation and identification of the calculated amount of protein in a single follicle lumen. In a recent work from this laboratory a qualitative analysis of thyroid proteins by microdisc electrophoresis was presented and a method for quantitative evaluation of the separation patterns described (5). The present paper is a preliminary report of the protein composition in colloids collected from single rat thyroid follicles.

Methods

Male rats, weighing 200-250 g, were reared at constant temperature and fed with standard fodder (iodine content: 20-30 ug/g pellet) and tap water ad lib. The animals were anaestetized with Mebumal, 80 mg/kg

body weight, and intubated. The thyroid was dissected free from surrounding tissue and the capsule was carefully removed from the isthmus of the gland. The follicles were punctured with a glass cannula, 5-15 u wide, with a sharpened tapered tip, and the extraction of the colloid was performed by negative pressure over the tip of the cannula achieved by a syringe which was adopted to the glass cannula by a plastic tube system. Prior to the puncture of the follicles the surface of the isthmus of the gland was dried by a warm air stream. In this way the hazard of contamination by superficial extracellular fluid was reduced. When the surface of the tissue was dry a few suitable superficial follicles were selected and punctured one by one. The duration of the extraction procedure was a few seconds. Leakage of blood into the follicles were readily seen; when a punctured follicle was filled with blood after the withdrawal of the glass cannula the extracted colloid was discarded. Immediately after extraction of the colloid the protein solution in the glass cannula was diluted with 0.2 µl cold phosphate buffered saline and stored for varying times at +4° C. The solution was then poured out of the cannula and layered on a 2.5% - 10% polyacrylamide gel system (pH 6.8) which was run at 20V-40V until the front had migrated 7 mm. With this technique the interval between the extraction of the colloid and the separation of its constituents can be kept down to a few minutes. In the present investigation the colloids were analysed within half an hour after the extraction. The separation patterns were recorded and the staining units calculated according to the methods earlier described (5). Since no determination of the sample volumes of the extracted colloids were performed on this material there are no values of the protein concentration in the colloids. The figures given represent the total amount of the extracted protein components. Due to the relatively small number of follicles analysed so far (11 follicles from 7 animals) no statistical analysis of the observations was performed.

Results

There were qualitative differences in the colloid composition between follicles of the same gland. And follicles with qualitatively similar protein compositions differed markedly with respect to the quantities of proteins. On quantitative basis two main types of follicles could be distinguished.

In the first type of follicles the main protein components of the colloid were found to be the 27S, 19S and 12S thyroglobulin units (Fig. 1). The relative amount of 19S varied between 64 and 81 per cent of the total pro-

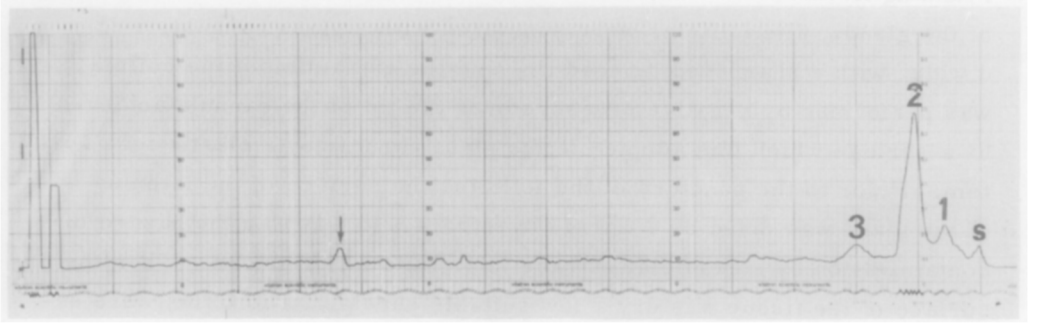

Fig. 1. Densitometric recording of disc electrophoresis pattern of the first type of colloid. S - starting point. 1 - 27 S; 2 - 19 S; 3 - 12 S. The arrow indicates a recording artifact.

tein whereas the 27S-unit always constituted less than 10 per cent. The 12S-unit varied between 14 and 32 per cent of the total protein and changed inversely to the 19S-unit. The total amount of protein was calculated to be about 8×10^{-3} µg per colloid (Table 1).

Table 1

Colloid No.	Protein fraction								Total prot. $\times 10^{-3}$ µg
	27 S $\times 10^{-3}$ µg	%	19 S $\times 10^{-3}$ µg	%	12 S $\times 10^{-3}$ µg	%	Albumin-like prot. $\times 10^{-3}$ µg	%	
1	0.5	4	7.0	64	3.5	32	-		11
2	0.7	9	5.7	75	1.2	16	-		7.6
3	0.5	7	4.8	70	1.6	23	-		6.9
4	0.3	5	5.2	81	0.9	14	-		6.4
5	0.4	6	3.5	51	2.5	36	<0.5	7	6.9
6	0.5	10	2.1	40	1.1	21	1.5	29	5.2
7	0.2	4	1.8	25	1.3	18	4.0	53	7.3

In some follicles with about the same amount of protein a component with a migration rate similar to that of serum albumin was found in addition to the three thyroglobulin units. In these follicles the relative amounts of 27S and 12S were largely the same as in the first type of follicles, 4-10 per cent and 18-36 per cent respectively, whereas the relative amount of the 19S-unit was markedly decreased, inversely correlated to the increased amount of the albumin-like protein fraction (Table 1).

In the second type of follicles, containing about ten times as much protein as the previous category, the albumin-like protein fraction was

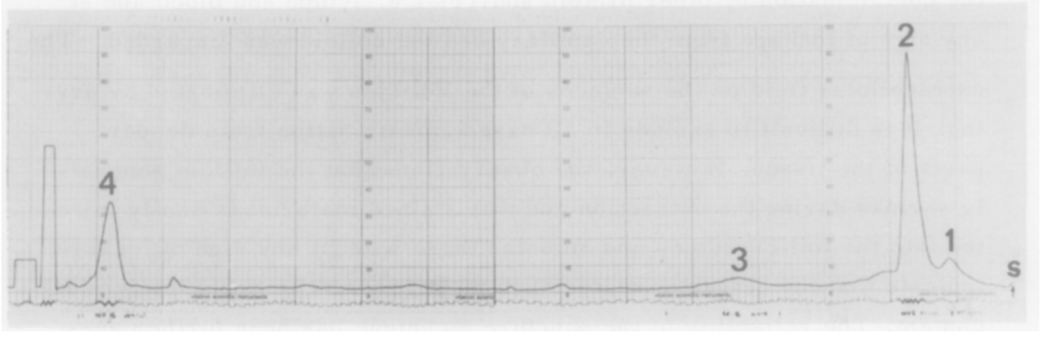

Fig. 2. Densitometric recording of disc electrophoretic pattern of the second type of colloid. S - starting point. 1 - 27 S; 2 - 19 S; 3 - protein with R_f: 0.27-0.35; 4 - Albumin-like protein.

Table 2

Colloid No.	Protein fraction								Total protein $\times 10^{-3}$ µg
	27 S $\times 10^{-3}$ µg	%	19 S $\times 10^{-3}$ µg	%	12 S $\times 10^{-3}$ µg	%	Prot. with R_f: 0.27-0.35	Albumin-like prot. $\times 10^{-3}$ µg	%
I	0.6	<1	10.5	12	-	-	+	~ 80	87	~90
2	3.3	<4	16	19	-	-	+	~ 65	77	~85
3	3.4	<4	40	45	+	-	+	~ 45	50	~90
4	-	-	18	15	1.6	<1	+	~100	85	~120

the predominant colloid constituent. In addition to this and the 27S, 19S and, in some cases, the 12S-unit, a protein which migrates between the 12S-unit and the albumin-like protein was found. The nature of this protein is not clear (Fig. 2 and Table 2).

Discussion

Micropuncture of thyroid follicles was originally introduced by DeRobertis for analysis of hydrolytic enzyme activity in the follicle lumen (6). A similar technique was used for microinjections into the follicle lumen by Seljelid (7). Recently, a study of the electrolytes in follicular lumina was performed on colloids obtained in mainly the same way (8). In the present investigation extreme care was taken in order to reduce the contamination by other protein sources i. e. lymph and blood and at any sign of leakage from the capillary bed the colloid was discarded. The extracellular fluid on the surfaces of the follicles was eliminated by drying. It is difficult to exclude in all cases contamination from deeper parts of the tissue. However, the observations that the follicle generally shrinks during the extraction and that stained material is easily injected into the follicle lumen and remains there without any sign of leakage, indicate that the extracted material in most cases represents the luminal content. The reliability of the puncture technique has been further analysed by Shagrin and Young (8).

Since more than one protein component was found in the extracted material the question whether the extracted colloid is representative of the total luminal content must be risen. Gersh and Baker (1) found that the colloid was homogeneous with respect to the U. V. absorbance. This observation, however, does not permit any conclusions about the distribution of the thyroglobulin units but indicates that the protein concentration is similar for all parts of the colloid. Probably, the whole colloid was extracted during the puncture of the follicle but this remains to be proved.

By means of direct analysis of the content in the follicular lumen it is possible to get new information of several aspects of thyroid physiology. As indicated from several works in this field every follicle probably constitutes a functional unit with a wide interfollicular variation of the colloid composition with respect to protein content (1). A recent investigation of the electrolytes in the follicular lumina also demonstrates a broad scattering in mainly the sodium concentration (8). The present observations confirm the heterogeneity of the colloids.

Whole gland experiments have demonstrated that the formation of 12S protein and thyroglobulin is microsome associated and that this fraction

contains newly synthesized protein before the supernatant fraction (9). Subfractionation of the microsomes has further revealed that ribosome-studded vesicles contain newly synthesized thyroglobulin prior to its appearance in smooth vesicles similar to those commonly seen in the apical region of the follicle cell (10). The interpretation of these findings is that thyroglobulin is mainly formed in the rough-surfaced endoplasmic reticulum and transferred to the follicle lumen. Although these observations represent a sum picture of the synthesis and transport between the epithelial cells and the lumina of all follicles in the gland it seems reasonable to assume that they illustrate the main mechanism for the synthesis and transfer of the proteins which are to be found in the follicle lumen.

The present preliminary observations confirm the view that thyroglobulin is transferred to the colloid as 19S generally represents the major constituent of the colloid proteins. In some cases, however, the relative amount of 19S is low and in this type of follicles the 4S component shows relatively high values. The physiological significance of this observation is not clear. However, Torresani et al. (11) reported that in rats with impaired synthesis of thyroglobulin a 4S albumin could be iodinated, and they suggested that this protein would act as the main substrate for thyroid hormone biosynthesis.

The high amount of 12S protein found in the colloid urges a closer investigation of the stability of this component and of 19S thyroglobulin. The short time interval between extraction and analysis of the colloid which is possible with the present technique is favourable for such a study which is now in progress.

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