LETTER TO THE EDITOR

T3-BINDING ALBUMIN IN HUMAN THYROID FOLLICULAR CELLS

The nature of the iodinated albumin-like protein which has been extracted from the thyroid gland is unknown.

The soluble iodinated albumin-like protein, which is distinct from thyroglobulin, may comprise from 2 to 13% of the iodoprotein in normal human and animal glands, and a much higher proportion under certain abnormal conditions (2, 3, 8).

Thyroid albumin contains MIT, DIT and hormones (T3, T4), whose ratios are quite different from those in thyroglobulin in which there is a high ratio of MIT and DIT and of iodothyrosines to iodothyronines (14).

Thyroid albumin is similar to albumin in its electrophoretic and ultracentrifugal characteristics. Although there is strong evidence that it is synthesized and iodinated in the thyroid gland, some points of the iodination process are still unclear (6, 13).

In this study, we tried to analyze the immunochemical characteristics of thyroid hormones which were contained in the thyroid albumin molecules (5, 10, 11).

Water soluble proteins of the thyroid tissues were utilized in this study. Normal thyroid tissue was obtained from a euthyroid patient who received a thyroidectomy for adenoma and the uninvolved tissue was used. Other samples were obtained from patients who underwent neck surgery for cancer of the larynx and who were free of clinical manifestations of thyroid disease.

Human thyroid albumin was fractionated by preparative flat-bed isoelectric focusing in a 3.5–10 pH gradient gel of Sephadex G-75 superfine with LKB 2117 Multiphor (15). Isoelectric focusing was performed at a constant 10 W for 16 hr at 10°C. The protein extracted from the gel in the range of pI 4.7–5.2 was separated again by polyacrylamide gradient gel electrophoresis (PAA 2/30), and the MW 67K fraction was tested immunochemically.

Immunodiffusion tests showed the thyroid albumin fraction to react only with anti-human albumin and with anti-T3 serum, but not with anti-thyroglobulin (19S) serum (Figs. 1-1, 1-2).

There was no precipitation reaction of the T3-free sample, prepared by charcoal absorption, with anti-T3 serum, but a precipitation line was observed with anti-albumin serum (Figs. 1-3, 1-4). The charcoal absorption method has been used to prepare T3-free serum by Larsen (7) and Mitsuma (12). In our study, 2 g of charcoal (Norit A, Sigma Chemical Co.) was added per 10 ml of human thyroid gland extract and incubated for 25 hr at 4°C. The mixture was centrifuged three times at 15,000 g and the supernatant was collected (9).

Anti-T3 serum was prepared according to Gharib, and the immunochemical specificities and sensitivities were tested by a radioimmunoassay (4).

The thyroid hormones remain in the bound form with thyroglobulin until secreted. The site of hydrolysis of thyroglobulin is a subject of dispute. Most of the evidence now supports the hypothesis that the thyroid cells ingest the colloid by pinocytosis, where the colloid merges with lysosomes. Thyroglobulin is known to

FIG. 1

FIG. 2

be broken by protease in the lysosomes and MIT, DIT, T3 and T3 are liberated into the cytoplasm. In our study, thyroid albumin was shown to be made up of albumin and T3. The liberated (free) hormones are considered to bind to albumin which is synthesized in the cells to make the thyroid albumin molecule. This is the same situation in T3-binding albumin in the serum, because this band was broken easily by charcoal absorption (1).

In the immunohistochemical study, we found T3 and albumin proteins in the follicle cells, not in the colloid. The data suggest that thyroid albumin results from the binding of the liberated hormones to albumin, as was suggested by the immunochemical data (Figs. 2-1, 2-2, 2-3).

In forth coming immuno-electron microscopical data, we would like to describe our research more clearly.

ACKNOWLEDGMENTS

The authors wish to thank Prof. Masaharu Horino, M. D., Division of Endocrinology, Department of Medicine, Kawasaki Medical School, for supply of the anti-T3, T4 serum. This investigation was supported in part by Kawasaki Medical School Project No. 57-308 and 57-309.

REFERENCES

1. Cody, V.: Thyroid hormones: Crystal structure, molecular conformation, binding, and structure-function relationships. *Rec. Progr. in Horm. Res.* 34; 437-475, 1981.
2. DeGroot, L. J. and Stanbury, J. B.: Hormone synthesis, secretion, and action. In the Thyroid and Its Diseases, 4th ed., A Medimedia Publication, Wiley Biomedical Division, John Wiley & Sons, New York, London, Sydney, Toronto, 1975, p. 37-109.
3. Falconer, I. R., Roitt, I. M., Seamark, R. F. and Torriginai, G.: Studies of the congenitally goitrous sheep. Iodoproteins of the goitre. *Biochem. J.* 117; 417-424, 1970.
4. Gharib, H., Ryan, R. J., Mayberry, W. E. and Hockert, T.: Radioimmunoassay for triiodothyronine (T3): I. Affinity and specificity of the antibody for T3. *J. Clin. Endocrinol. Metab.* 33;

FIG. 1-1. Immunochemical analysis on the human thyroid water soluble protein after isoelectric focusing against anti-total human thyroid gland rabbit serum. "S" is the water soluble fraction as a control, and wells 1-30 are filled with the extracted proteins after isoelectric focusing. Antiserum was filled in the groove far 4 mm from the well lines. Thyroglobulin (Tg) was localized in the area of PI 4.2 (fr. 4)-5.5 (fr. 12), albumin (Alb) in PI 4.7 (fr. 7)-5.2 (fr. 10) and the PI range of 7.0 (fr. 19)-7.5 (fr. 21) was suggested to be an enzyme fraction (E).

FIG. 1-2. Immunochemical analysis on the human thyroid water soluble protein after isoelectric focusing against anti-T3 rabbit serum. The T3 area is localized in PI 4.7 (fr. 7)-5.2 (fr. 10). Abbreviation is the same in Fig. 1-(1).

FIGS. 1-3, 4. Microoachterlony test on human thyroid water soluble fraction without (3) and with charcoal absorption (4) against anti-total human thyroid gland rabbit serum (A.HT(T)S), anti-T3 rabbit serum (A.T3S), anti-human serum rabbit serum (A.HS.S) and anti-human serum albumin rabbit serum (A.Alb.S).

FIGS. 2-1-3. Immunohistochemical studies are shown on the normal human thyroid gland against anti-T3 rabbit serum (1) and anti-human serum albumin rabbit serum (2 and 3). It shows the positive grain reactions anti-human serum albumin and anti-T3 serum to be located in the follicular cells, but the colloid is negative. It suggests that the thyroid albumin is the liberated hormones which is bound with albumin, as we described in immunochemical data. · 800

- 509-516, 1971.
5. Ikeda, A., Mishima, N., Horino, M. and Harada, T.: Immunochemical studies of human thyroid albumin and T3. In The 25th Japan Endocrine Society, May, 1982 in Tokyo Thyroid Session, 1982, p. 11 (abstract).
 6. Jonckheer, M. H. and Karcher, D. M.: Thyroid albumin. I. Isolation and characterization. *J. Clin. Endocrinol. Metab.* 32; 7-17, 1971.
 7. Larsen, P. R.: Direct immunoassay of triiodothyronine in human serum. *J. Clin. Invest.* 51; 1939-1949, 1972.
 8. Lissitzky, S., Codaccioni, J.-L., Bismuth, J. and Depieds, R.: Congenital goiter with hypothyroidism and iodo-serum albumin replacing thyroglobulin. *J. Clin. Endocrinol. Metab.* 27; 185-196, 1967.
 9. Matsumura, S., Oyama, H., Horino, M., Yamaguchi, T., Kobayashi, K. and Suetsugu, N.: Radioimmunoassay for triiodothyronine. *Kawasaki Med. J.* 1; 15-25, 1975.
 10. Mishima, N., Ikeda, A. and Harada, T.: Immunochemical studies on water soluble proteins of human thyroid gland. In The 25th Japan Endocrine Society, May, 1982 in Tokyo Thyroid Session, 1982, p. 11 (abstract).
 11. Mishima, N., Ikeda, A., Yoshii, I. and Ugawa, A.: Immunochemical studies on water soluble proteins of normal human thyroid gland. *Igaku-no-ayumi* 126; 551-554, 1983.
 12. Mitsuma, T., Colucci, J., Shenken, L. and Hollander, C. S.: Rapid simultaneous radioimmunoassay for triiodothyronine and thyroxine in unextracted serum. *Biochem. Biophys. Res. Commun.* 46; 2107-2113, 1972.
 13. Otten, J., Jonckheer, M. and Dumont, J. E.: Thyroid albumin. II. *In vitro* synthesis of a thyroid albumin by normal human thyroid tissue. *J. Clin. Endocrinol. Metab.* 32; 18-26, 1971.
 14. Torresani, J., Roques, M., Peyrot, A. and Lissitzky, S.: Misen en evidence, purification et propriétés d'une iodoalbumine, constituant physiologique de la glande thyroïde de rat. *Acta Endocrinol.* 57; 153-157, 1968.
 15. Winter, A., Perlmutter, H. and Davies, H.: Preparative flat-bed electrofocusing in a granulated gel with the LKB 2117 Multiphor. *LKB Application Note* 198, 1975.

July 25, 1983
and in revised form
August 13, 1983

AKIRA IKEDA, NOBORU MISHIMA, RYO
KAWANO AND TANEKAZU HARADA
*Department of Anatomy,
Division of Endocrine Surgery and
Department of Surgery,
Kawasaki Medical School, Kurashiki 701-01*