THYROID PEROXIDASE-INDUCED THYROID HORMONE SYNTHESIS IN RELATION TO THYROGLOBULIN STRUCTURE

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1 Introduction

The native structure of thyroglobulin (Tgb) has been shown to be important in the synthesis of thyroid hormones [1–4] Its alteration by denaturing agents such as urea or guanidine considerably decreases or even abolishes the yield of $T_4 + T_3$ following iodination of the protein in vitro both chemically and enzymatically Thyroglobulin preparations used in these experiments were obtained from glands frozen before the purification process, conditions which were shown later to produce limited proteolytic attack of the protein, by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of the reduced protein [5] Such limited proteolysis has no detectable influences on certain properties of the protein such as sedimentation coefficient, solubility in salts and electrophoresis in the absence of reducing agent However, since hormone formation is the consequence of iodination of some tyrosine residues of thyroglobulin into iodotyrosines, followed by their subsequent coupling into iodothyronine residues, and since it is likely that a limited number of hormone forming sites are present in the protein [2,6–8], it is probable that interruption of the continuity of the chain by peptide bond cleavage would influence the 3-dimensional arrangement of hormone forming sites and would result in a decreased capacity of the protein to synthesize thyroid hormones We demonstrate here that within the range of iodine concentrations normally observed in human thyroglobulin, thyroid hormone synthesis obtained by thyroid peroxidase-catalyzed iodination of very poorly iodinated human thyroglobulin is considerably decreased when limited proteolysis is observed in the protein

Abbreviations Tgb, thyroglobulin, MIT, 3-monoiodotyrosine, DIT, 3,5-diiodotyrosine, T_3, 3,5,3′-triiodothyronine, T_4, thyroxine

2 Materials and methods

2 1 *Thyroglobulin preparations*

Undegraded poorly iodinated thyroglobulin (Tgb-1) was purified from human goiters (colloid or familial goiters) in the conditions of [5,9] The thyroid tissue was obtained at operation, immersed in cold (0°C) 0 1 M sodium phosphate (pH 7 2), sodium azide 0 02% and used within 1 h for Tgb purification by slice extraction, precipitation at 1 8 M sodium phosphate (pH 7 2) and filtration on Biogel A-5 m in 0 05 M sodium phosphate (pH 7 2) Fractions corresponding to 19 S were pooled, dialyzed against water and freeze-dried Another type of Tgb preparation (Tgb-2) was obtained from a frozen goiter using slice extraction and DEAE-cellulose chromatography in phosphate–NaCl buffers (pH 6 4) of increasing ionic strengths [10] Iodine content of the Tgb preparations used was 0 01–0 04% (g/100 g) iodine

2 2 *Thyroglobulin iodination*

The reaction was carried out in 1 ml final vol 0 05 M sodium phosphate (pH 7 2) containing 1 nmol thyroglobulin, 20–200 nmol $K^{125}I$, 3 μmol glucose and 1 unit (5 μg) thyroid peroxidase The reaction

was started by addition of 4 μg glucose oxidase, continued for 15 min at 37°C and stopped by $NaHSO_3$ addition. Iodide was eliminated from the reaction mixture by filtration on Sephadex G-25 columns (0.9 × 15 cm) equilibrated with 0.05 M sodium phosphate (pH 7.2). The amount of iodine incorporated into Tgb was calculated on the basis of the ratio of radioactivity in the fraction excluded from the gel to the total radioactivity eluted from the column. In all these experiments, iodination was performed at 37°C for 15 min. Incubation was not prolonged beyond that time in order to avoid artefactual attack by proteolytic enzymes possibly contaminating the preparation. Incubation of the iodination mixture up to 90 min increased the $T_4 + T_3$ yield by only 10–15% as compared to 15 min incubation.

2.3. *Gel electrophoresis*

Polyacrylamide gel electrophoresis (4% acrylamide total concentration and 4% methylenebisacrylamide relative to acrylamide) was performed in 0.05 sodium phosphate (pH 7.2) containing 0.1% SDS [11]. Samples (10–15 μg) were dissolved in this buffer and electrophoresed for 5 h at 8 mA/tube. Staining with Coomassie blue and destaining were done according to [12].

2.4. *Others*

Peroxidase was solubilized from bovine thyroids according to [13] and purified on Sephacryl S 200 and Biogel HTP according to [14]. The specific activity of the preparation used was 205 U/mg protein, one unit being defined as the quantity of enzyme required to oxidize 10 μmol iodide/min under standard conditions [14]. Protein concentration of Tgb solutions was determined according to [15] using bovine serum albumin as standard and iodoamino acid distribution in Tgb was estimated by ion exchange chromatography in a Technicon Autoanalyzer according to [16] after digestion with pronase and leucylaminopeptidase.

3. Results and discussion

When analyzed by SDS–polyacrylamide gel electrophoresis, both Tgb preparations obtained from fresh (Tgb-1) or frozen (Tgb-2) glands showed a similar pattern, with a predominant 12 S band and a limited number of faster migrating species (fig.1). After reduction with DTT the band pattern of Tgb-1 remained almost unchanged while that of Tgb-2 showed the presence of fast migrating material representing 30–40% of the total material applied to the gel (fig.1). The disimilarity of structure between Tgb-1 and Tgb-2 was revealed by the reduction of the intrachain disulfide bonds which despite limited proteolysis, maintained the subunits as a single molecular species.

In spite of identical sedimentation behavior in the ultracentrifuge, both types of Tgb preparations behaved differently towards enzymatic iodination. Tgb-2 was shown to be a poorer substrate than Tgb-1

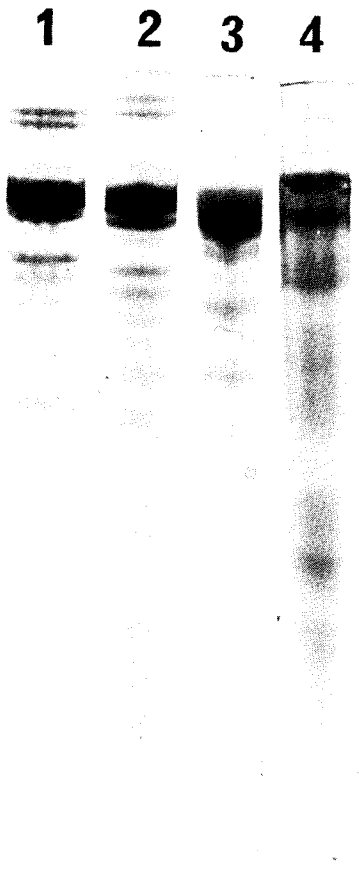

Fig.1. SDS–polyacrylamide gel electrophoresis of poorly iodinated human thyroglobulin preparations purified from fresh (Tgb-1) or frozen (Tgb-2) goiters. Tgb-1, without (1) and after (3) reduction; Tgb-2, without (2) and after (3) reduction.

for thyroid hormone synthesis following iodination catalyzed by thyroid peroxidase At levels of iodination in the range (0 2–0 4%) of those encountered in glands of humans with normal iodine intake, the yield in iodothyronines formed from Tgb iodination was 2–3-times higher in Tgb-1 than in Tgb-2 (fig 2) In 5 experiments, at iodination levels of 25 iodine atoms/mol Tgb (0 48% iodine), Tgb-1 contained on a molar basis 3-times more $T_4 + T_3$ (1 45 ± 0 08 (mean ± SE) mol/mol Tgb) than Tgb-2 (0 55 ± 0 04)

The better efficiency of undegraded Tgb-1 to support hormone synthesis is also obvious when the distribution of iodine between iodoamino acid residues is considered On the basis of 1 T_4 residue

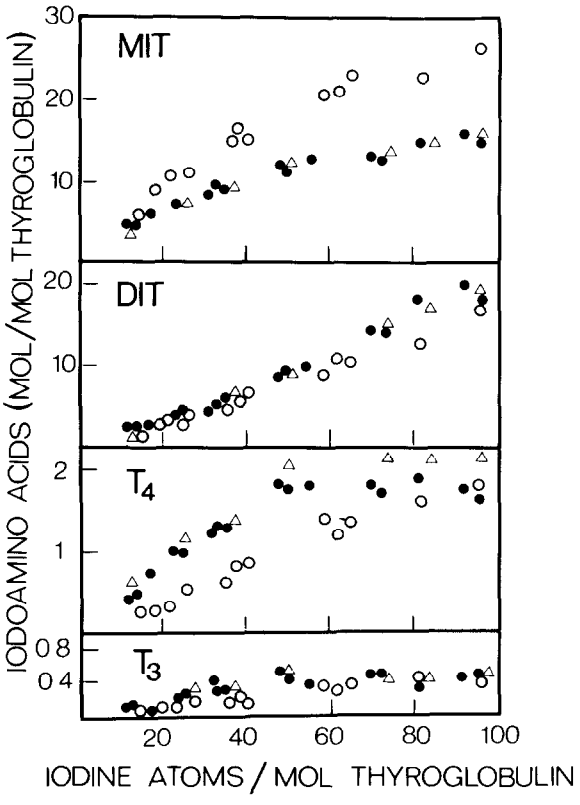

Fig 2 Iodoamino acid content of 2 types of human goiter thyroglobulins iodinated in the presence of thyroid peroxidase and H_2O_2 as a function of the number of iodine atoms incorporated per mol protein (•) Tgb-1, (○) Tgb-2, (△) Tgb-1′ Tgb-1′ was obtained by direct Biogel A-5 m gel filtration of the slice extract The top 19 S peak fraction was used without further treatment for iodination In this preparation, sodium azide was excluded from all buffers

resulting from the coupling of 2 DIT residues, and T_3 resulting from the coupling of 1 DIT and 1 MIT, it has been estimated that at 25 iodine atoms/mol protein, 2 6 mol DIT out of 6 5 coupled into iodothyronines in Tgb-1 representing a yield of hormone formation of 40%, whereas only 1 mol DIT in 4 5 participated in the coupling reaction in Tgb-2, i e , a 22% yield

From 50 iodine atoms incorporated/mol Tgb-1, the amount of $T_4 + T_3$ reached 2 2 mol/mol protein, then plateaued at this value up to iodination levels of 100 iodine atoms/mol In Tgb-2, the number of $T_4 + T_3$ residues increased continuously, reaching 2 2 mol/mol protein only at ~100 iodine atoms/mol Tgb (fig 2) At all iodination levels, the iodine distribution in iodotyrosines was different in the two kinds of preparations The number of MIT/mol protein was higher in Tgb-2 than in Tgb-1 whereas the reverse was observed for the total number of DIT residues (taking into account estimated DIT and the amount derived from the residues which coupled into iodothyronines) Such a situation has been shown to reflect Tgb denaturation Indeed, at alkaline pH or in the presence of denaturing agents, chemical iodination of poorly iodinated human Tgb resulted in higher relative amounts of iodotyrosines and especially of MIT than in their absence or at neutral pH, whereas $T_4 + T_3$ formation was markedly decreased [3] The same inverse relation between numbers of iodotyrosines and derived iodothyronines is observed when goiter Tgb iodinated in vitro in the presence of thyroid peroxidase is compared to Tgb iodinated in vivo (reviewed in [17]) Since iodine distribution in iodoamino acids is independent of iodination mechanisms, it is concluded that for a given Tgb iodine content a higher number of iodotyrosine residues formed reflects a less ordered conformation of the protein resulting in a decreased aptitude of thyroid hormone formation To date it is probable that denatured Tgb preparations have been used for iodination studies Our experiments which compare the peroxidasic iodination of an undegraded or almost undegraded preparation of human goiter Tgb with a preparation showing limited proteolytic attack clearly demonstrate that the better the preservation of the native structure of the protein the higher its hormonogenic capacity In the range of normal iodine content, even limited proteolysis of Tgb results in a

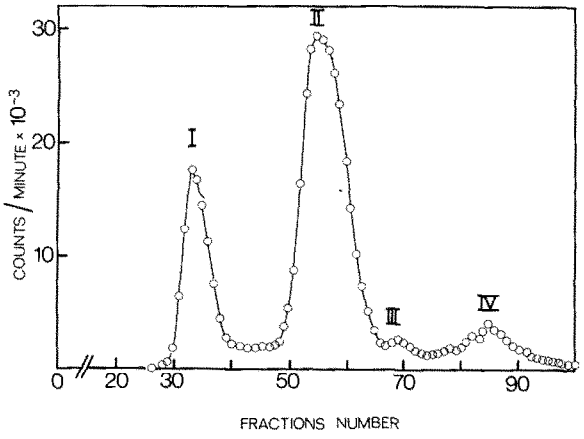

Fig.3. Sepharose 6B gel filtration of human goiter 19 S thyroglobulin iodinated by thyroid peroxidase. Iodine content was 24 iodine atoms/mol Tgb. Sepharose 6B column (0.9 × 55 cm) was equilibrated with 0.05 M sodium phosphate (pH 7.2) containing 0.1% SDS. Fractions of 1 ml were collected. (I) ⩾ 27 S species; (II) 19 S; (III) 12 S; (IV) material < 12 S.

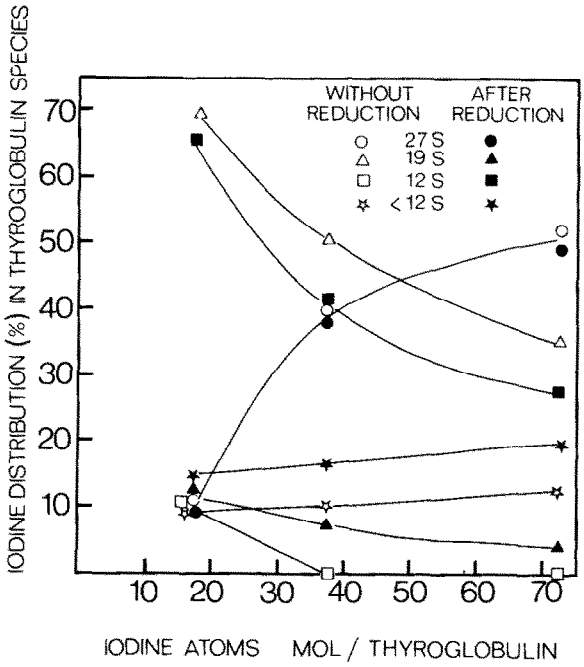

Fig.4. Distribution of iodinated thyroglobulin species in human goiter thyroglobulin (Tgb-2) iodinated in the presence of thyroid peroxidase as a function of iodine content of the protein. Samples of iodinated Tgb were analyzed by Sepharose 6B gel filtration before (open symbols) or after reduction (closed symbols) with 1% dithiothreitol.

large decrease of thyroid hormone synthesis in vitro.

To ascertain that the difference in hormone forming capacity of the 2 types of Tgb preparations was not due to further attack during iodination by protease possibly contaminating the preparations, iodinated Tgbs were analyzed by SDS–polyacrylamide gel electrophoresis in the same conditions as uniodinated proteins. Only a small fraction of the material submitted to electrophoresis was detected in the gels, suggesting that most of the iodinated material had not penetrated into the 4% gels used. Indeed, Sepharose 6B gel filtration of the iodinated material in 0.05 M sodium phosphate (pH 7.2) containing 0.1% SDS showed the presence of iodinated species >19 S (⩾27 S) (fig.3) whose proportion increased with the iodination level of Tgb to reach 60% of total iodoproteins for 72 iodine atoms incorporated/mol Tgb (fig.4). Interestingly, this material of very large size was insensitive to dissociation into species which dissociated almost completely into 12 S (fig.4). Absence of dissociation of thyroid peroxidase-induced polymers (⩾27 S) in the presence of SDS and reducing agents suggests that covalent linkages are involved in the polymerization process, in agreement with similar findings described in lactoperoxidase catalyzed iodination of hog 19 S Tgb [18]. The considerable extent of polymerization of 19 S Tgb detected in vitro is not observed in vivo since 27 S and larger polymers represent only 5–15% of soluble thyroid iodinated proteins in several species. Covalent association of 19 S molecules in Tgb polymer (⩾27 S) formed in vitro and probably resulting from oxidative modifications of some amino acid residues are likely to produce alterations of the 3-dimensional conformation of the constituent peptide chains and subsequent reduction in the hormone-forming capacity of the in vitro-formed polymers as compared to Tgb iodinated in vivo. Indeed, the maximum number of T_4 residues synthesized in vitro under thyroid peroxidase-catalyzed iodination of human goiter Tgb was ⩽2–2.5 residues/mol 19 S Tgb [8] whereas it tends to reach 4 mol T_4/mol 19 S Tgb in in vivo Tgb iodination at iodine contents from 0.01–0.7% in human Tgb [2] and from 0.16–1.10% in procine Tgb [6].

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