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Iodine induced alteration in immunological and biochemical properties of thyroglobulin

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The influence of iodine-iodide solution on the biochemical and immunological properties of human thyroglobulin (hTg) were studied. Human Tg preincubated with the iodine-iodide solution is split to small molecular mass fragments after disulphide bridge reduction with dithiothreitol. The peptide bond cleavage by iodine pretreatment and reduction is possibly linked with the coupling reaction of diiodotyrosyl residues. Pretreatment of hTg with iodine-iodide solution at 1 - 10 μM decreased the binding of autoantibodies to hTg. The iodine-iodide induced inactivation of hTg autoepitopes is pH dependent and is possibly caused by iodination of tyrosyl residues present in the epitope structure.

Thyroglobulin (Tg)[1], the iodoglycoprotein dimer (*m* 660 kDa) of the thyroid gland, is the matrix within which the thyroid hormones are synthesized [1, 2]. Tyrosine residues in Tg are iodinated by thyroid peroxidase and diiodotyrosine residues are coupled by the same enzyme with protein bound thyronine residues. During hormone formation the coupling reaction involves the fission of a diiodotyrosine residue and transfer of the diiodophenolic moiety to the second (acceptor) diiodotyrosyl residue [3]. At the donor side an unstable moiety of dehydroalanine is formed and called by some authors the "lost side chain". Some authors suggested that during the coupling reaction a peptide bond cleavage occurs at the dehydroalanine site [4]. Indeed, the presence of the 26 kDa hormone-rich peptide was found after reduction of human Tg [4 - 6].

Iodine can iodinate tyrosyl residues in Tg and catalyse the coupling reaction of diiodotyrosine moieties with thyronines [7]. We have studied the effect of iodine on immunological properties of human Tg, and tried to test the hypothesis on the peptide bond cleavage in Tg.

MATERIAL AND METHODS

Purification of human Tg. Crude human thyroglobulin was isolated from thyroid glands obtained after strumectomy of Graves' goitre as described by Derrien & Roche [8]. It was then purified by affinity chromatography on Sepharose 4B-concanavalin A column as described by Gardas [9].

Pretreatment with iodine of Tg and other proteins immobilized on polystyrene plates. Polystyrene plates were coated with thyroglobulin (or lactoperoxidase, human IgG or BSA, 2 μg/ml) overnight at 20°C in 0.1 M sodium bicarbonate solution containing 2 mM sodium azide. The freshly coated plate was washed twice with 0.9% NaCl containing 0.1% Tween

[1]Abbreviations: BSA, bovine serum albumin; e.l.i.s.a., enzyme-linked immunosorbent assay; h.p.l.c., high performance liquid chromatography; *m*, molecular mass; Tg, thyroglobulin

20 and twice with distilled water. The thyroglobulin coated plate was preincubated with iodine-iodide solution (molar ratio of iodine to iodide 1:4) at a concentration from 0.1 to 50 μM in appropriate buffer for 30 min at 20°C. The following buffers were used: 0.1 M sodium carbonate-bicarbonate buffer, pH 9.0; 0.1 M sodium bicarbonate solution, pH 8.0; and phosphate-buffered saline, pH 7.0.

Assay of the reaction of Tg-autoantibodies with immobilized Tg. Plates coated with Tg with or without iodine pretreatment, were incubated for 60 min with pooled serum from thyroid autoimmune disease patients, containing Tg-autoantibodies (18000 IU/ml). The serum was diluted 2000-fold with the phosphate-buffered saline containing 0.05% Tween 20 and 0.1% BSA. The plates were washed 3 times with the same solution, and the amount of autoantibodies bound to Tg was determined by the reaction of rabbit antihuman IgG antibody conjugated with horse-radish peroxidase by e.l.i.s.a.

H.p.l.c. separation of thyroglobulin and other proteins. Thyroglobulin, BSA, and cytochrome *c* (2 mg/ml) in 0.1 M sodium carbonate buffer, pH 9.6, were incubated with 1 mM iodine-iodide solution for 30 min. After iodine pretreatment Tg or other proteins were analysed by h.p.l.c. (Shimadzu) on the Superose 12 or Superose 6 Pharmacia column in 20 mM Tris/HCl buffer containing 0.5 M NaCl and 0.05% sodium deoxycholate. In some experiments thyroglobulin was reduced with 10 mM dithiothreitol for 30 min before chromatography. The h.p.l.c. column was calibrated by molecular mass standard proteins obtained from BioRad (Austria).

Sera and reagents. Sera obtained from the Warsaw Outpatients Department of Endocrinology, were screened for Tg antibodies by e.l.i.s.a. Sera containing high antibody titre were pooled, treated with sodium azide (5 mM) and stored at –20°C. The pooled serum used contained 18000 IU/ml of thyroglobulin autoantibodies. Horse-radish peroxidase conjugated antibodies were obtained by the periodate method as described by O'Sullivan & Marks [10]. Lactoperoxidase was obtained from Calbiochem (Switzerland), gliadin from Sigma (U.S.A.). Polystyrene plates were obtained from High Tech. Lab. (Warsaw, Poland). Other reagents, of the highest purity available, were bought through POCH (Gliwice, Poland).

RESULTS

Preincubation of human thyroglobulin immobilized on polystyrene plates with iodine-iodide solution decreased Tg ability to bind human anti-Tg autoantibodies from pooled serum of patients with thyroid autoimmune diseases (Fig. 1). The decrease in autoantibodies binding was dependent upon iodine concentration and pH. At alkaline pH (9.6) the Tg autoantibodies binding sites were inactivated by 50% after preincubation with 1 μM iodine. Thyroglobulin pretreated with iodine at pH 7.0 was also inactivated, but at higher iodine concentration (Fig. 1). Pretreatment of human IgG with iodine, did not change the reaction with rabbit antibodies at iodine concentration up to 200 μM. Iodine pretreatment of lactoperoxidase at concentration higher than 50 μM increased the reactivity with appropriate rabbit antibodies. Iodine pretreatment of gliadin did not change its reaction with human antibodies from patients with coeliac disease.

Iodine treatment of thyroglobulin did not change its migration rate on the Superose 12 column (Fig. 2) and Superose 6 column (not shown) during h.p.l.c. chromatography. Human Tg reduced with 10 mM dithiothreitol migrated as a 330 kDa protein on the Superose 6 column, as predicted (not shown). During chromatography on Superose 12, Tg and its subunits obtained after reduction were not separated and were eluted in the void volume of that column (Fig. 2). Iodine-treated and reduced thyroglobulin was separated during chromatography on the Superose 12 column into three peaks; 1, the native protein; 2, a broad peak of mean molecular mass 100 kDa and 3, a small peak of 25 kDa.

DISCUSSION

Low concentrations of iodine at alkaline pH induce changes in the thyroglobulin molecules leading to their decreased reactivity with autoantibodies from patients with thyroid autoimmune diseases. The mechanism of iodine induced changes in the thyroglobulin molecule is

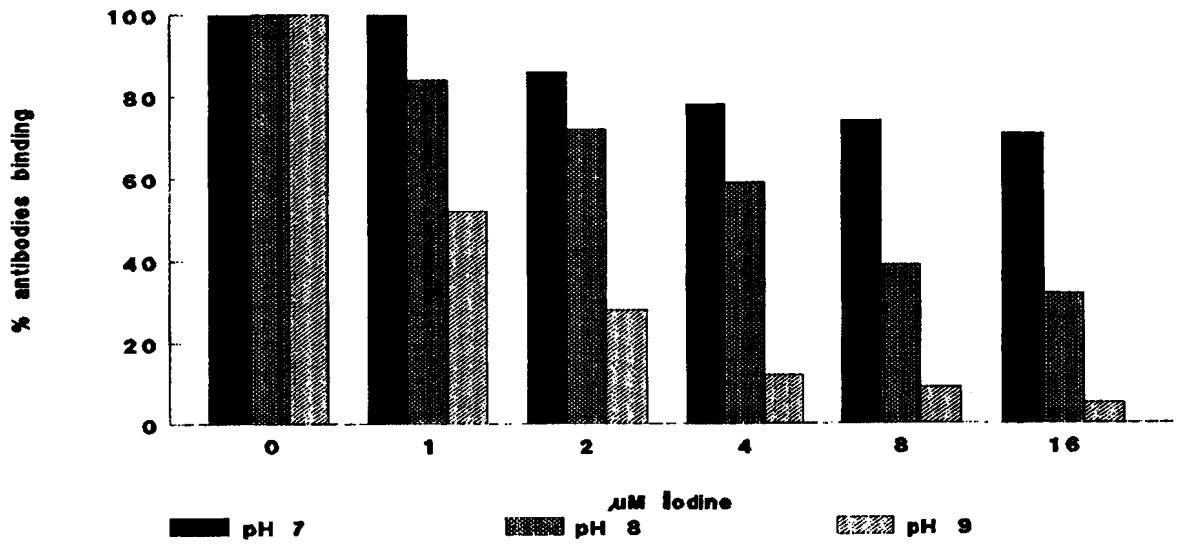

Fig. 1. The effect of iodine pretreatment of polystyrene bound human thyroglobulin on its ability to bind autoantibodies.
Binding of antibodies to thyroglobulin is expressed in relation to control (without pretreatment) taken as 100

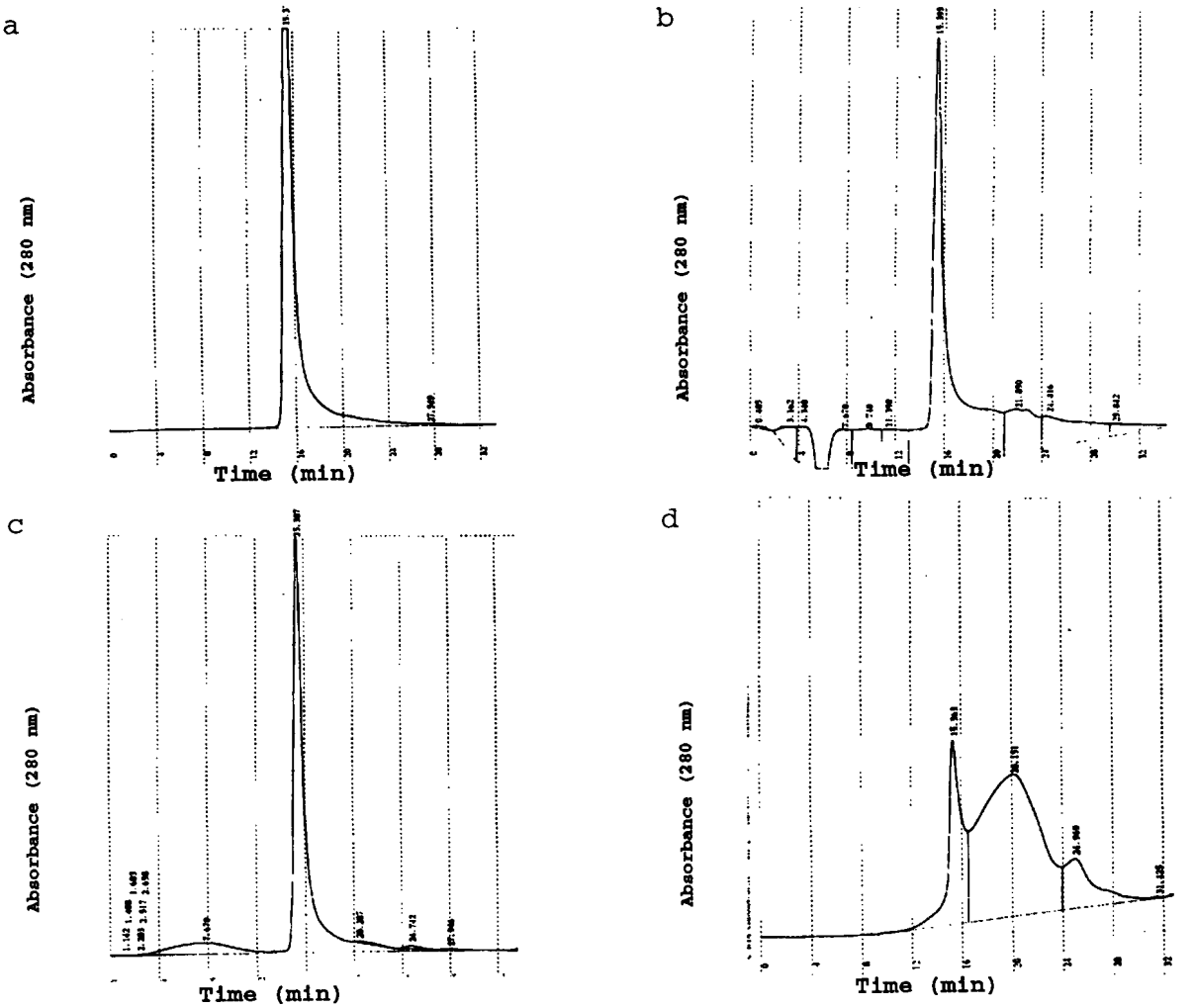

Fig. 2. H.p.l.c. chromatography of human thyroglobulin on the Superose 12 column.
a, Native; b, after dithiothreitol reduction; c, after iodine pretreatment; d, after iodine pretreatment and reduction with dithiothreitol

not yet explained; several possibilities can be considered. Oxidation, intramolecular coupling, and denaturation processes could be responsible for the decreased immunological activity after iodine pretreatment. Iodine-induced changes in the thyroglobulin molecule are unlikely to be specific for that glycoprotein. However, pretreatment of human IgG, lactoperoxidase and gliadin with iodine at much higher concentrations did not lead to decreased reactivity with appropriate antibodies. It is possible that iodination of tyrosine residues, which are present in Tg epitopes recognized by autoantibodies, decreases the affinity of the latter to the modified antigens. This possibility needs further experimental evidence.

The iodine-treated thyroglobulin after dithiothreitol reduction is split to smaller fragments of a mean molecular mass about 100 kDa. Peptide bonds are very stable and it is difficult to assume that the treatment with iodine under mild conditions could lead to the cleavage of peptide bonds due to direct interaction with iodine. Besides, iodine pretreatment of albumin or cytochrome *c* did not lead to any changes in the molecular mass measured before and after reduction. Peptide bond cleavage in thyroglobulin has been considered by Marriq *et al.* [4] as part of the diiodotyrosine coupling reaction. Our current hypothesis is that the iodine-induced peptide bond cleavage in thyroglobulin is the result of following reactions:

- 1, iodination of tyrosine to diiodotyrosine residues;
- 2, iodine catalysed coupling of diiodotyrosine residue with concomitant formation of dehydroalanine residues at the donor sites;
- 3, cleavage of peptide bonds next to the unstable dehydroalanine residues at alkaline pH during dithiothreitol reduction.

The presented results support the suggestion of Marriq *et al.* [4] that peptide bond cleavage occurs during the coupling of diiodotyrosine residues in thyroglobulin and thyronine formation. The more significant peptide bond cleavage during iodine catalysed coupling in comparison to enzymatic coupling may be due to the low specificity of the non-enzymatic reaction. The coupling catalysed by thyroid peroxidase is highly specific and only a few molecules of thyroid hormones are formed [1]. A Tg molecule contains 120 tyrosine residues [1] and it is probable that more tyrosine residues are coupled by iodine catalysed coupling and, as a result, more peptide bonds are split at the donor sites.

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