Characterization of the human thyroid microsomal antigen involved in thyroid autoimmunity

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Autoimmune disorders of the human thyroid gland are associated with the presence of auto-antibodies to components of the gland such as thyroglobulin, the thyroid microsomal/microvillar antigen, a second colloid antigen and the receptor for thyroid-stimulating hormones (Volpe, 1981). The thyroid microsomal/microvillar antigen has been shown to be expressed in intracellular membranes and on the apical surface of follicular cells (Khoury et al., 1981, 1984). Under certain circumstances, this auto-antigen is accessible to the complement fixing, anti-microsomal auto-antibody and may lead to the destruction of the gland. In this report, some biochemical characteristics of the microsomal/microvillar antigen, identified by immunoprecipitation with patients' sera, are presented.

Serum from patients with thyroid disorders diagnosed as thyrotoxicosis, Hashimoto's disease and primary myxoedema containing auto-antibodies to thyroglobulin and/or microsomal antigen were utilized; normal human serum was used as a control together with affinity-purified rabbit anti-(human thyroglobulin). Thyroid microsomes were prepared from glands removed during surgery, as described by Roitt et al. (1964), and solubilized in 1% sodium deoxycholate in 10 mM-Tris/HCl, pH 7.9, containing 1 mM-phenylmethylsulphonyl fluoride and 1.25 mM-iodoacetamide. Radioiodination of the solubilized membrane was accomplished

![Fig. 1. Analysis by SDS/polyacrylamide-gel electrophoresis and autoradiography of immunoprecipitates with radioiodinated thyroid microsomal membrane and patients sera](image-url)

Thyroid microsomal membrane prepared from a Graves disease gland was radio-labelled after solubilization in sodium deoxycholate. After pre-clearing of non-specific binding material with normal human serum and *Staphylococcus aureus*, the labelled lysate was divided into seven equal aliquots for immunoprecipitation with various sera/antibodies. The serum sample number of the patient and the titre of auto-antibodies as ascertained by Thymune-T and Thymune-M kits is mentioned. TGHA and MCHA refer to titres of anti-thyroglobulin and anti-microsomal antibodies respectively. RaTg refers to affinity purified rabbit anti-(human thyroglobulin); as negative controls the second step antibody was omitted (lane C) or substituted with normal human serum (lane NHS). The gel consists of a 5-15% polyacrylamide gel, electrophoresed under reducing conditions. The numbers to the left of the gels represent $M_r \times 10^{-3}$. Tg refers to the major polypeptide band of thyroglobulin at 260000 $M_r$ and Mic. Ag. to the microsomal antigen at 105000 $M_r$. 

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Abbreviation used: SDS, sodium dodecyl sulphate.
by a modification of the chloramine-T procedure, followed by G-25 chromatography. Immunoprecipitations were performed as described by Banga et al. (1984) using formalin-fixed \textit{Staphylococcus aureus} to isolate the antigen–antibody complexes, which were analysed by polyacrylamide-gel electrophoresis in SDS.

The composition of the immunoprecipitates with various sera, under reducing conditions, are shown in Fig. 1. The polyclonal antibody to thyroglobulin (lane RzTg) identifies thyroglobulin migrating with an apparent $M_r$ of 260,000 plus weaker bands at 290,000 and 142,000. In contrast, sera from patients containing both anti-thyroglobulin and anti-microsomal antibody (lanes 3599 and 3353) identify the thyroglobulin polypeptide at 260,000 $M_r$, plus an additional band at 105,000 $M_r$. This band was also present in sera containing anti-microsomal antibodies only (lane 2337 and 4882) and no thyroglobulin polypeptides were visible. The 105,000-$M_r$ band was not visualized with the polyclonal anti-thyroglobulin precipitate (lane RzTg) nor with normal human serum (lane NHS) or where antibody was omitted (lane C); a number of non-specific bands were identified with the two last-named controls and a strong band migrating with the tracking dye was prominent in all the lanes. Although not shown in Fig. 1, immunoprecipitations with human sera containing anti-thyroglobulin antibodies only precipitated the thyroglobulin-specific polypeptides but not the 105,000-$M_r$ component. Immunoprecipitations with anti-microsomal antibodies on vectorially radiiodinated thyroid follicular cells have given concordant results, with the cell-surface peptide also proving to have an $M_r$ of 105,000. Furthermore, the presence of intrachain disulphide bonds within this molecule is indicated, as assessed by a small shift in mobility in SDS/polyacrylamide-gel electrophoresis under non-reducing conditions, to an $M_r$ of 117,000 (not shown).

The results suggest that the thyroid microsomal antigen is a protein, which is probably stabilized with internal disulphide bonds and which is distinct from membrane-bound thyroglobulin. The intracellular and cell surface forms of this antigen are of a comparable $M_r$, and studies can now be initiated to study the function of this molecule in the thyroid gland.

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Activation of carbonic anhydrase III by active-site modification

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All established functions of the zinc metalloenzyme carbonic anhydrase (EC 2.4.1.1) are related to its ability to rapidly catalyse the reversible hydration of CO$_2$. In addition it acts as a general acid–base catalyst, with much lower turnover numbers towards several other substrates such as pnitrophenyl acetate (Lindskog et al., 1971). In reptiles, birds and mammals, three genetically distinct isozymes, CA I, CA II and CA III, have been characterized (Tashian et al., 1983). CA III, which has been found in red muscle fibres of several species, has much lower activity both towards CO$_2$ and p-nitrophenyl acetate (Koestet et al., 1981) and is relatively insensitive to inhibition by certain sulphonamides which are potent inhibitors of CA I and CA II (Maren & Sanyal, 1983).

Koster et al. (1981) have demonstrated a low acid phosphatase activity in CA III from several species. This suggests that this isozyme may have a physiological role different from, or additional to, that of CO$_2$ hydration. Sequencing studies on human and bovine CA III indicate a remarkably different active-site structure from those of CA I and CA II and of the five putative active-site residues unique to CA III, two, at positions 67 and 91, are arginine (Tashian et al., 1980a). Tashian et al. (1980b) have reported enhancement of the bicarbonate dehydration activity of CA III by modification with the arginine-modifying reagent, 2,3-butanedione.

Bicarbonate dehydration was measured using a pH-stat assay system (30mM-NaHCO$_3$, pH 7.1, 2°C) and esterase activity towards p-nitrophenyl acetate (1mM) was measured spectrophotometrically, both as described by Chegwidden et al. (1984).

Carbonic anhydrase isoenzymes were prepared as described by Carter et al. (1984). Modification with 2,3-butanedione in 50mM-borate, pH 8.3, was performed in the dark at 25°C, conditions shown to selectively modify arginine residues (Borders & Riorden, 1975).

On modification with 2,3-butanedione, a marked activation occurred of both the bicarbonate dehydration and esterase activities of human and bovine CA III but of only the esterase activity of chicken CA III. CA I and CA II remained unaffected by similar treatment (Table I).

Enhancement of the bicarbonate dehydration activity of human CA III was biphasic, a rapid, initial activation of 70–100% being followed by a slower process giving >200% after 24h.

Tryptic digestion and sequence analysis were performed on human CA III (56μM) after modification with 2.5mM-butanedione at 25°C (A) for 10min and (B) for 45min. (A) showed only partial modification of Arg-91, whilst (B) showed modification of arginine residues at positions 39, 67, 80 or 89 and 91. Arginine is also present at position 89 in CA I and CA II which were not activated and is not present at position 39 in bovine CA III which was activated. Residue 80, which may have been modified, is not at the active site. This suggests that activation is associated with modification of one or both arginine residues at positions 67 and 91.

Abbreviation used: CA, carbonic anhydrase.