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The Subunit Structure of Thyroglobulin

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Human and rat thyroglobulin were reduced and alkylated in aqueous alkaline conditions in the absence of denaturants; the product of reduction in both cases has been found to have mol.wt. about 165000, or one-quarter that of the native molecule.

Thyroglobulin is a heterogeneous glycoprotein whose principal constituent has a mol.wt. of 660000 and sedimentation coefficient of 19S; minor constituents (27S and 31S) represent about 5% of the total protein.

Many attempts have been made in the past decade to determine the subunit structure of thyroglobulin after reduction and alkylation of its 120 disulphide bonds. Pierce *et al.* (1965) reduced human thyroglobulin in urea solution and obtained a single component with an apparent mol.wt. in the range 110000–125000, by sedimentation velocity. In the following year, de Crombrughe *et al.* (1966) reduced calf thyroglobulin in urea or guanidinium chloride and estimated the mol.wt. of the product to be approx. 165000, also by sedimentation velocity. Lissitzky *et al.* (1968) concluded that the principal product of reduction of sheep thyroglobulin in 8M-urea had mol.wt. 80000.

Subsequently, analysis of the reduction products of thyroglobulin by polyacrylamide-gel electrophoresis has revealed a multiplicity of fragments, which probably results from the inherent instability of reduced thyroglobulin even at -20°C , as first noted by Pierce *et al.* (1965) and confirmed by Charlwood *et al.* (1970). This instability is due to autolysis of thyroglobulin subunits by a factor that, so far, has not been separable from the thyroglobulin molecule during its purification (Ambesi-Impiombato & Pitt-Rivers, 1971; Pitt-Rivers, 1975; Rolland & Lissitzky, 1976). Nevertheless, a general pattern has emerged of rather large subunits from reduced thyroglobulin; Nissley *et al.* (1969) were able to separate and purify two major components from their reduction products of bovine thyroglobulin with mol.wts. one-quarter and one-half that of the native protein. Ui & Sorimachi (1975, 1976) have shown that, when pig thyroglobulin is reduced at pH 8 in 6M-guanidinium chloride, the product obtained consists essentially of a single subunit with mol.wt. 165000. However, a different finding has been obtained by Lissitzky *et al.* (1975), who

reduced thyroglobulin from sheep and pig thyroid follicles reconstituted *in vitro*, and consistently obtained a subunit of mol.wt. 310000, or half that of the native protein. These workers suggest that the smaller subunit is obtained only after preliminary freezing and thawing of the thyroid tissue, with possible liberation of the proteolytic factor referred to above (Rolland & Lissitzky, 1976).

It should be noted that thyroglobulins from different species may have different subunit structures. Spiro (1973) obtained three principal fractions from reduced guinea-pig thyroid; these have since been shown (Haeberli *et al.*, 1975a) to have mol.wts. of 295000, 210000 and 110000; further, the proportion of these subunits obtained by reduction varies inversely with their iodine content (Haeberli *et al.*, 1975b).

Materials and Methods

Thyroglobulin

Thyroglobulin was obtained from fresh or frozen human thyroid or pooled frozen rat thyroids. The tissue was gently disrupted in a glass homogenizer in 2 vol. of buffer, pH 8.7, containing 17 mM-Tris, 50 mM-glycine and 0.02% NaN_3 . The suspension was filtered through gauze and centrifuged at 3000g. The supernatant was passed through a Sephadex G-200 column equilibrated with the same buffer, and the protein emerging at the front was analysed in 4.25% Cyanogum gels (BDH Chemicals, Poole, Dorset, U.K.) in the same buffer. The 19S thyroglobulin was the principal band, with small amounts of 27S and 31S protein. Any preparations showing fractions running faster than 19S were discarded.

Reduction and alkylation

The thyroglobulin solution (1–2 mg/ml) was adjusted to pH 10.5 with 0.2M-NaOH (cf. Mehta & Rose, 1974) and reduced with dithiothreitol (20 mol/mol of SH groups) for 30 min at 20°C . The pH was then adjusted to 8.5, and the product was alkylated with iodoacetamide (1.2 mol/mol of SH groups) for 15 min. Excess of alkylated dithiothreitol was removed by dialysis (two 1 h periods, volume ratio 1000:1).

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Polyacrylamide-gel electrophoresis

Cyanogum gels, 3% and 4% (5% of methylenebisacrylamide to acrylamide), were made up in the Tris/glycine buffer, pH 8.7, containing 4M-urea and 0.1% sodium dodecyl sulphate in 11cm×0.5cm tubes (cf. Russell & Skehel, 1972). The gels were not layered with water or buffer to give a flat surface, since it was found that even the slightest dilution at the top of the gel enhanced the apparent mobility of the heavier fractions. The running buffer contained 0.1% sodium dodecyl sulphate but no urea; samples (20–100 µg) were treated with sodium dodecyl sulphate and urea in excess over the content of the gel buffer and transferred to the gel under the running buffer. A current of 0.5mA/cm of gel was used. Electrophoresis was generally stopped when the front marker in the sample, Bromophenol Blue, neared the bottom of the gel. The gels were fixed in 7% (w/v) formic acid, stained in 0.1% Coomassie Brilliant Blue in 7% (v/v) acetic acid containing 30% (v/v) of methanol and de-stained in 7% (v/v) acetic acid. For mol.wt. determinations, the following (unreduced) markers were used: ovalbumin (44000); bovine serum albumin monomer (68000); transferrin (76000); bovine serum albumin dimer (136000); thyroglobulin (660000), which partially decomposes in urea to give a subunit of 330000.

Results

The results of two such reductions are shown in Plate 1.

It is seen that human and rat thyroglobulins yield essentially a single subunit, occasionally with a suggestion of doublet formation; this doublet appearance has also been noted by Rolland & Lissitzky (1976) in chains of reduced pig and sheep thyroglobulins.

Estimation of the molecular weights by the method of Weber & Osborn (1969) gave values between 160000 and 170000.

Comment

In the present conditions, namely in the absence of denaturant, it is unlikely that more than 50% of the disulphide bonds of thyroglobulin will be reduced (de Crombrughe *et al.*, 1966); nevertheless it appears that all the inter-chain disulphide linkages can be broken in these conditions, contrary to the finding by Ui & Sorimachi (1975, 1976), who considered that the presence of urea or guanidine was necessary for complete reduction.

I consider that an essential condition for success is speed, and analysis of reduction products should be carried out immediately after reduction; storage at –20°C, even for a week, inevitably leads to breakdown of the original subunits and the appearance of multiple bands on the analytical gels. A similar instability

has been found in the reduction products of adenovirus components (Pereira & Skehel, 1971). Nevertheless, though it is recognized that the mol.wts. here obtained are not definitive, the present findings are in agreement with those of Ui & Sorimachi (1975, 1976), namely that the subunit of thyroglobulin represents one-quarter of the native molecule. In this connexion it is noteworthy that Vassart *et al.* (1975) have described the synthesis *in vitro* of a peptide related immunologically to thyroglobulin with mol.wt. approx. 30000. The discrepancy between my findings and those of Lissitzky *et al.* (1975) and of Vassart *et al.* (1975) cannot at present be explained.

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EXPLANATION OF PLATE I

Electrophoresis in sodium dodecyl sulphate/urea/4% polyacrylamide gels of the reduction products of human and rat thyroglobulins

Key: H, human; R, rat. The numbers indicate the positions and mol.wts. of the markers run in a separate experiment.