THE NATURE OF THE BREAKDOWN OF THYROGLOBULIN

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Received 16 August 1971

1. Introduction

Thyroglobulin consists chiefly of a component of M.W. 660,000 and sedimentation coefficient 19 S. On complete reduction of the 101 disulphide bonds, subunits are obtained (TgM) with M.W. ca. 165,000 [1]. However, these slowly give rise to products of lower M.W. [2] even when stored at -20° [3]. In a previous paper from this laboratory [4] we described the slow breakdown of TgM at pH 8.7 in the presence of SDS, leading to the formation of two products, B and C, with M.W. 35,000 and 20,000 respectively. In the present communication we will describe further breakdown products, their composition and conditions which favour their formation.

2. Materials and methods

Pig 19 S Tg was prepared by polyacrylamide gel electrophoresis according to Brownstone [5]. Reduction and alkylation in 8 M urea or 6 M guanidinium chloride was carried out as previously described [4]. After removal of denaturant and excess reagents by dialysis against running tap water, the reduction product was then dialysed against buffer at various pH, alone or containing SDS, urea or guanidinium chloride.

Abbreviations:
SDS: sodium dodecyl sulphate
Tg : thyroglobulin
TgM: thyroglobulin monomer
DFP: diisopropylfluorophosphate

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All solutions in which TgM was stored contained 0.02% NaN_3. After 18–22 days at room temperature the solutions were freed of denaturants and brought to pH 8.7. SDS was added equivalent to 1:1 by weight of protein. The breakdown products were analysed by gel filtration through a Sephadex G-200 column 2 × 120 cm in 0.05 M glycine and 0.0165 M Tris, pH 8.7, containing 0.03% SDS, 0.02% NaN_3 and 0.1 M NaCl (R buffer). The protein peaks were monitored in an LKB Uvicord II before separation in a fraction collector. Maximum loading was 120 mg total protein. The fractions were freed from SDS and buffer by repeated dialysis against 1 mM NH_3 and lyophylized. Molecular weights were estimated according to Yphantis [6], iodoamino acids according to Edelhoch [7], hexoses according to Roe [8], sialic acid according to Aminoff [9], disulphide bonds according to Ellman [10] and SDS according to Pitt-Rivers and Ambesi Impiombato [11].

3. Results and discussion

The breakdown products of TgM as separated by gel filtration are shown in fig. 1.

Fraction A1 represents TgM and other fractions excluded from the gel. (Polyacrylamide gel electrophoretic analysis of freshly prepared A1 in R buffer has shown A1 to be heterogeneous.) Fraction A2 did not separate satisfactorily from A1 but was isolated only when all A1 had disappeared. The molecular weights of A3, B, C and D are 90,000, 35,000, 20,000 and 2500, respectively. All fractions bound SDS to protein in a ratio of 1.4:1 except D which

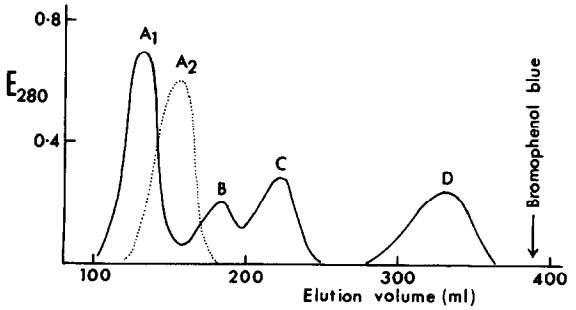

Fig. 1. Elution pattern on Sephadex G-200 of protein fractions obtained by the breakdown of TgM. Peak A2 only appears occasionally among these products.

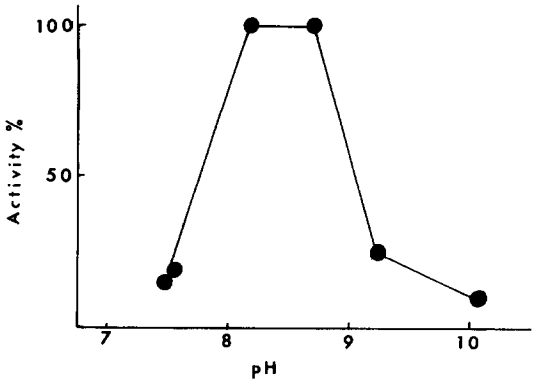

Fig. 2. Effect of pH on the breakdown of TgM, expressed as percentage disappearance of fraction A1.

bound no SDS. Fractions B and C had amino acid contents, hexose (4%) and sialic acid (1.2%) indistinguishable from those of Tg (see [12, 13]). The mono- and di-iodotyrosine contents of B and C were the same as that of Tg but the thyroxine content of C was consistently higher (0.96%) and that of B, lower (0.4%) than that of Tg (0.6%).

The amino acid content of fraction D closely resembled that of Tg except for the iodoamino acids which were absent. These, if present as free amino acids in fraction D were presumably lost during prolonged dialysis before lyophilization. D contained 1.4% of iodine which would represent only a fraction of a molecule of monoiodotyrosine. The hexose content of D was 5%.

The pH optimum of TgM breakdown is shown in fig. 2, and lies between 8.2 and 8.7.

TgM breakdown was inhibited, though not entirely arrested by SDS (SDS:protein, 1:1), 8 M urea and 6 M guanidinium chloride. Together, these findings suggested that breakdown might be due to proteolysis.

It was noted that after compelte breakdown of TgM, when only fractions C and D remained, gel analysis always revealed a small amount of material with absorption at 28C ım at the void volume of the column (F1). Occasionally a small peak (F2) was seen immediately behind F1. These fractions were freed from SDS and concentrated at pH 8.7 by vacuum dialysis. The concentrated solutions were spotted onto a thin gelatin film according to Pickford and Dorris [14]. After 4 hr exposure in a moist atmosphere, fraction F1 was found to have liquified the gelatin; this is shown in fig. 3. Fraction F_1 also converted B to C and D. Enzymic activity towards gelatin and other added substrates could not be demonstrated until the native substrate had been entirely digested. Fraction F2 had no demonstrable proteolytic activity.

Attempts to inhibit proteolysis by treatment of the reduction product with DFP, photooxidation in the presence of methylene blue or by heating for 7 min at 100° in 6 M guanidinium chloride at pH 1 and pH 8.7 were all unsuccessful.

This suggests that the enzyme must be very firmly bound to Tg and TgM.

19 S Tg, if stored at pH 8.7 at room temperature, for several weeks, broke down to give fractions appearing on gel filtration in the same positions as B and C. Addition of not more than 1–2 mg SDS per ml of reaction mixture accelerated breakdown, indicating that a certain degree of unfolding favours enzymic action. The unreduced B and C fractions were analysed for disulphide bonds; in both, the S-S content was 1%, which is the same as that in 19 S Tg (101 S-S per 660,000). The breakdown does not therefore involve the rupture of disulphide bonds.

Breakdown of TgM was not inhibited when the experiments were carried out under completely sterile conditions, and even in long term experiments, lasting several months, there were no signs of bac-

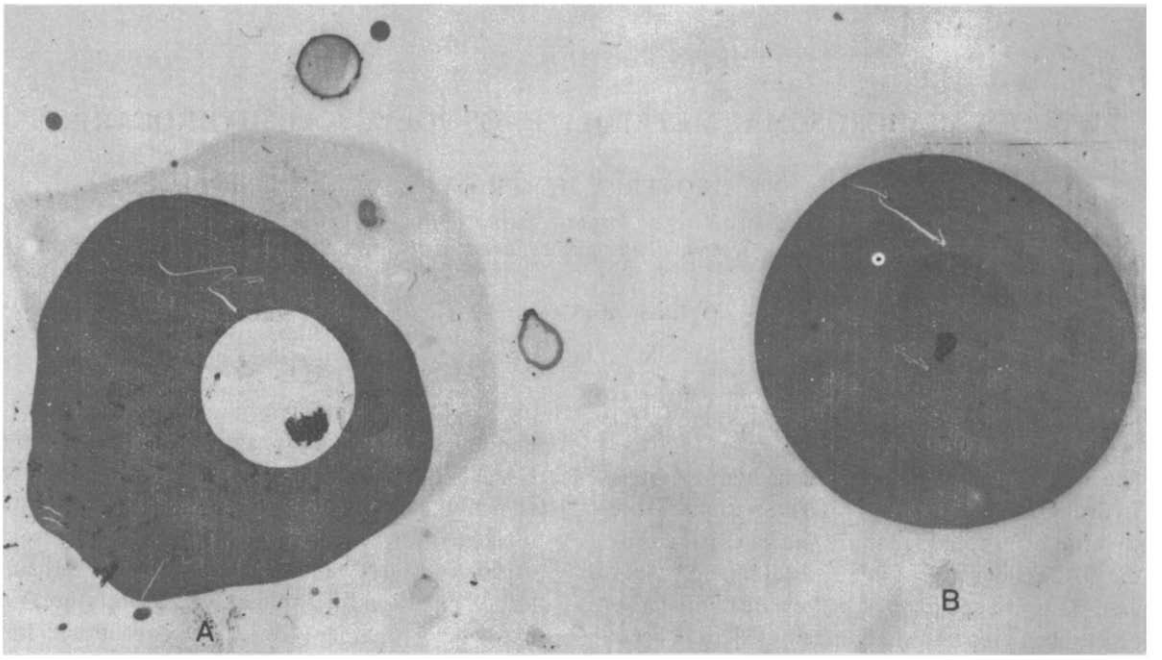

Fig. 3. Effect of fraction F1 (spot A) and buffer control (spot B) on gelatin (Ilford gelatin plates coated for electrofocussing). The protein was stained with Harris's haematoxylin.

terial contamination. We conclude that during the preparation of 19 S Tg, a proteolytic enzyme becomes irreversibly bound to it and is responsible for its subsequent breakdown, either in reduced or unreduced state. Alternatively the enzyme may constitute an inherent part of the Tg molecule.

Acknowledgements

We thank Dr. P.A. Charlwood for the molecular weight determinations. F.S.A.I. was in receipt of a Fellowship Award under the European Science Exchange Programme between the Accademia Nazionale dei Lincei and the Royal Society.

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