GUINEA PIG THYROGLOBULIN: MOLECULAR WEIGHT OF SUBUNITS AND AMINO ACID AND SUGAR COMPOSITIONS

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A reevaluation of the molecular weights of the three major subunits of guinea pig thyroglobulin has been undertaken in gels of varying concentration. Since the apparent molecular weights determined from plots of relative mobility versus log molecular weight were found to decrease as the gel porosity decreased, the relationship of retardation coefficient to molecular weight was used. Molecular weights obtained by this technique were 192,000, 131,000, and 121,000; these were close to the values obtained from electrophoresis in 8% gels. Amino acid and sugar compositions are also reported; the guinea pig protein, like that from human thyroids, contains galactosamine in addition to glucosamine, suggesting that it contains three types of saccharide units. Aminoterminal analyses performed by dinitrophenylation indicated nonstoichiometric amounts of aspartic acid, leucine and serine.

INTRODUCTION

In a previous report dealing with the subunit composition of thyroglobulins from a number of species (1) it was shown that the protein from the guinea pig differs from all the others studied. In contrast to the marked subunit heterogeneity of most thyroglobulins, that from the guinea pig had three main components, the molecular weights of which were calculated to be approximately 215,000, 175,000 and 140,000. More recently, an investigation of this protein carried out in another laboratory (2) reported subunit molecular weights of 295,000, 210,000 and 110,000. Since this matter is of importance both to an understanding of the architecture of the molecule and to an interpretation of biosynthetic data, we have performed new studies utilizing as molecular weight markers protein polymers which have become available from commercial sources and which have considerably higher molecular weights than those employed in the past.

Also included in this presentation are the results of compositional studies of the guinea pig protein dealing with its amino acids, sugars and aminoterminal residues.

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MATERIALS AND METHODS

Preparation of thyroglobulin - Guinea pig thyroids obtained from Pel-Freez (Rogers, Arkansas) were sliced thinly and extracted with 0.85% NaCl at 2° for 2 hours. The soluble protein obtained by centrifuging at 100,000 x g for 1 hour was fractionated between 1.47 and 1.68 M potassium phosphate, pH 6.8 (3), dissolved in 0.1 M phosphate buffer, pH 7.0, and applied to a Bio-Gel A-5m column (1). The protein peaks were pooled, dialyzed 36 hours at 2° against several changes of distilled water and lyophilized.

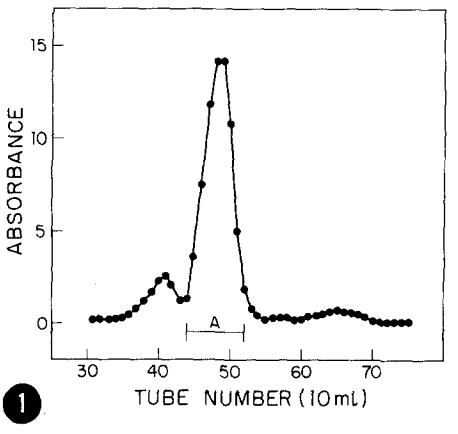
Analytical techniques - Amino acid analyses were performed on a Technicon Amino Acid Analyzer (140 cm column) after hydrolysis in constant boiling HCl for 24 hours at 105° under nitrogen. Amino sugars were determined on the Technicon NC-2 Analyzer with a pH 5 gradient (4) after hydrolysis in constant boiling HCl for 16 hours at 105° under nitrogen. Neutral sugars were quantitated on the Technicon Sugar Analyzer (5) after hydrolysis in 1 N HCl for 5 hours and desalting of the hydrolysates on coupled columns of Dowex 50 and Dowex 1 (6). Sialic acid was measured by the resorcinol procedure (6).

Protein determinations were carried out by the Lowry method (7); dinitrophenylation, hydrolysis and identification of dinitrophenyl amino acids were accomplished as previously described (8). The values obtained for amino sugars, neutral sugars and dinitrophenyl amino acids were corrected for destruction observed when standard compounds were subjected to the same conditions of hydrolysis and analysis.

Gel electrophoresis - Sodium dodecylsulfate (SDS)-polyacrylamide electrophoresis was performed according to the procedure of Weber and Osborn (9) at 4.5 mamp per gel. Acrylamide concentrations varying from 3 to 8% were employed and the columns used were 17 cm in length to facilitate measurement of high molecular weight components in less porous gels. The thyroglobulin samples and the standard proteins were heated in 1% SDS-5% mercaptoethanol at 100° for two minutes prior to electrophoresis. Marker proteins used included myosin (mol. wt. 200,000), bovine serum albumin (66,000), ovalbumin (46,000), phosphorylase b (94,000) and polymers prepared by BDH Chemicals, Ltd., which represented a protein monomer (mol. wt. 53,000) through hexamer. Gels were stained with Coomassie blue by the procedure of Fairbanks, et al. (10) and were scanned at 550 nm in the gel attachment of the Gilford spectrophotometer. For each standard protein and subunit, plots of the log of the R_f (relative mobility measured with respect to the midpoint of the added Brom phenol blue) versus gel concentration were prepared and from these Ferguson plots the slope (retardation coefficient, K_R) and intercept (log Y_o) were determined as described by Rodbard (11). Linear plots and approximately equal intercepts were obtained from the thyroglobulin subunits and most of the standard proteins using the data obtained from the 3 through 7% gels. For the BDH tetramer and pentamer, however, the data from the 3 through 6% gels were used because of the difficulty of measuring these slow components accurately in the 7% gels. Molecular weights of the guinea pig subunits were determined from a plot by the method of least spares of the K_R versus molecular weights of the standard proteins. In addition, molecular weights were obtained at each gel concentration from a least squares plot of the R_f of the BDH standards versus log molecular weight.

RESULTS

Upon filtration on Bio-Gel A-5m of the protein precipitating between 1.47 and 1.68 M potassium phosphate, 83% emerged in peak A (Fig. 1), which in the



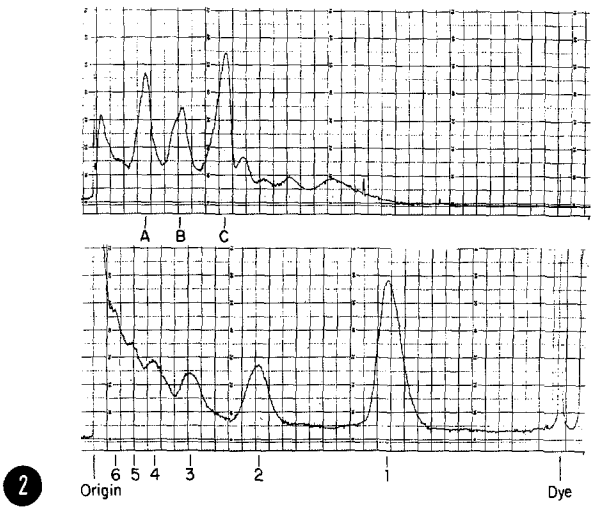


Fig. 1. Filtration of guinea pig thyroid protein precipitating between 1.47 and 1.68 M potassium phosphate, pH 6.8, on a Bio-Gel A-5m column (4.0 x 69 cm) in 0.1 M phosphate buffer, pH 7. The absorbance obtained in the Lowry reaction (7) is plotted.

Fig. 2. Densitometry of stained gels after polyacrylamide-sodium dodecyl-sulfate electrophoresis. In the lower frame are shown the marker proteins (monomer through hexamer) while the upper frame contains the scan of guinea pig thyroglobulin (50 µg). The guinea pig protein has in addition to the main components, A, B and C, which represented 36, 22 and 29% of the total, other bands for which molecular weights of 117,000, 102,000, 91,000 and 72,000 were calculated. These latter components contributed 4.6, 1.5, 2.3 and 4.3% of the absorbance of the guinea pig protein, respectively. In both gels the baseline is elevated near the origin by a small amount of protein which did not penetrate the gel.

case of calf thyroglobulin has been shown to correspond to the 19 S protein (1), 11% eluted earlier (corresponding to 27 S molecules) and the remainder was found in smaller molecular weight components. Peak A was utilized for the investigations reported here.

Polyacrylamide electrophoresis - The behavior of the three major subunits of guinea pig thyroglobulin during electrophoresis in 5% gels is shown in Fig. 2 in comparison to the BDH polymers. The apparent molecular weights obtained from plots of R_f versus log molecular weight decreased markedly as gel concentration increased (Table I). Similar behavior has been observed for the larger subunits of calf thyroglobulin (1). Methods which have been proposed

Table I. Variation of guinea pig thyroglobulin subunit apparent molecular weight[a] with gel concentration

Subunit[b]	Gel Concentration					
	3%	4%	5%	6%	7%	8%
	molecular weight x 10^{-3}					
A	400	269	240	221	190	188
B	260	199	193	188	153	158
C	180	144	155	155	132	126

[a] Determined from plots obtained by the method of least squares of R_f versus log molecular weight of the BDH monomer (53,000 daltons) through pentamer.

[b] The subunit designations are the same as those in Fig. 2.

to determine correct molecular weights under these circumstances include performing electrophoresis in increasingly concentrated gels until the fall in molecular weight becomes asymptotic (12) and utilizing the relationship between retardation coefficient and molecular weight, which is independent of gel concentration (11). A plot of the retardation coefficients determined for the standard proteins is shown in Fig. 3. The molecular weights calculated for subunits A, B and C from this graph were 192,000, 131,000 and 121,000 (the 95% confidence limits calculated for the line were $\pm$ 16,200). These values are close to those determined in the 8% gels (Table I), suggesting that the method of Segrest and Jackson (12) is a valid approach to this problem.

Composition - The amino acid composition of the guinea pig protein (Table II) appears similar to those of the thyroglobulins from calf, sheep, pig and man (8). Like these other proteins it contains approximately 200 disulfide bonds per molecule and is rich in glutamic acid, leucine and serine. The present data are in agreement with analyses reported by Haeberli, et al. (2). Dinitrophenylation studies indicated that aspartic acid, serine and leucine occupy terminal positions on peptide chains in this protein (Table II), but

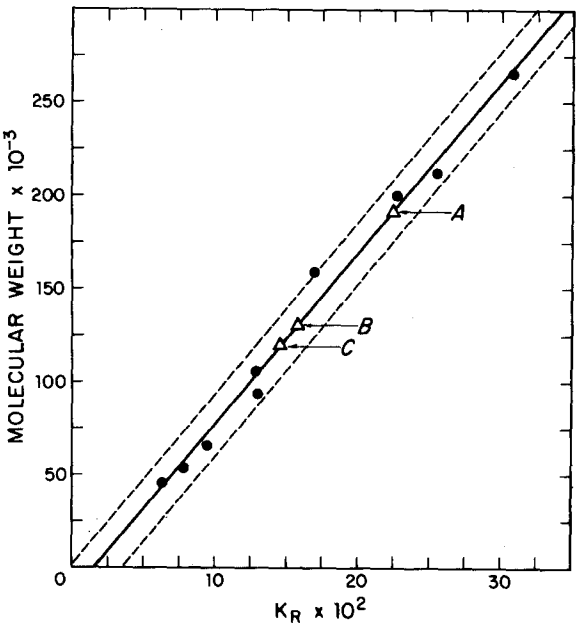


Fig. 3. Plot (solid line) of K_R versus molecular weight obtained by the method of least squares for several standard proteins (•). The molecular weights of the proteins used and the log Y_o values obtained from the Ferguson plots were as follows: ovalbumin, 46,000, 0.146; BDH monomer, 53,000, 0.214; bovine serum albumin, 66,000, 0.234; phosphorylase b, 94,000, 0.221; BDH dimer, 106,000, 0.217; BDH trimer, 159,000, 0.214; myosin, 200,000; 0.320; BDH tetramer, 212,000, 0.510; and BDH pentamer, 265,000, 0.614. The K_R values for the guinea pig thyroglobulin subunits A, B and C are shown (Δ). The area within the dashed lines indicates the 95% confidence limits of the line.

as has been found for all other species studied, these amino acids are not present in stoichiometric amounts (8).

The most interesting aspect of the sugar composition (Table III) is the presence of galactosamine, which has also been found in human thyroglobulin where it is a component of a carbohydrate unit (unit C) linked to serine (threonine) residues (13) as well as in a glucuronic acid-containing carbohydrate unit (14). Since the guinea pig protein was found devoid of glucuronic acid (14) its galactosamine is likely to be present in a type C unit. The high ratio of mannose to galactose in the guinea pig protein indicates that it also contains the two types of asparagine-linked units (units A & B) previously found in the thyroglobulins from other species (15).

Table II. Amino acids and NH_2-terminal amino acids of guinea pig thyroglobulin

Component	Residues/1000 total amino acids	Residues/molecule[a]
Aspartic acid	77.6	383
Threonine	52.5	259
Serine	95.4	472
Glutamic acid	133.1	658
Proline	72.1	356
Glycine	80.0	385
Alanine	79.7	394
Valine	58.4	289
Half-cystine	38.4	190
Methionine	14.5	72
Isoleucine	24.3	120
Leucine	97.2	480
Tyrosine	23.6	116
Phenylalanine	52.7	260
Lysine	30.5	147
Histidine	15.1	76
Arginine	56.6	279
DNP-aspartic acid[b]		1.5
DNP-serine		0.59
DNP-leucine		0.45

a. A molecular weight of 670,000 was used for this calculation.
b. DNP refers to the 2,4-dinitrophenyl derivative.

Table III. Sugar composition of guinea pig thyroglobulin

Component	mg/100 mg protein[a]	Residues/molecule
Galactose	1.2	50
Mannose	2.5	104
Fucose	0.32	15
Glucosamine	2.9	97
Galactosamine	0.77	25
Sialic acid	1.6	38
Total	9.3	329

a. Expressed as residue weight. The amino sugars are calculated as the N-acetyl form.

DISCUSSION

The nature of the biosynthetic subunits of the thyroglobulin molecule has been a matter of controversy for a number of years. It is generally agreed that the native protein has a molecular weight of 670,000 (16) and that it can dissociate under mild conditions to yield the half-molecule (17). After reduction of disulfide bonds, however, a complex pattern is observed by polyacrylamide electrophoresis in SDS and molecular weights of approximately 200,000 have been estimated for the major bands (1). The exact size of these subunits has been difficult to assess, both because the molecular weights calculated from gel electrophoresis vary with gel concentration and because of a lack, up to now, of suitable high molecular weight markers.

Two recent studies have calculated molecular weights of approximately 300,000 for the largest subunit of guinea pig (2) and pig (18) thyroglobulin and proposed that this represents the intact biosynthetic subunit of the protein. In both of these studies, part of the evidence provided was based on a comparison by gel filtration and gel electrophoresis of reduced thyroglobulin subunits with unreduced standard proteins. Tanford and his colleagues (19) have reported differences in the radii of reduced and unreduced proteins in denaturing solvents and have cautioned against the use of unreduced molecules as standards for unknowns in the reduced state in studies dealing with gels.

Haeberli, et al. (2) provided additional evidence for a molecular weight close to 300,000 from sedimentation equilibrium studies. Although this method of molecular weight determination is generally given a high degree of credence, its weak point in a study of reduced molecules in denaturing solvents is an uncertainty with respect to the correct partial specific volume to be employed.

In the present study, the use of the BDH polymers which retain their high molecular weight in the presence of reducing compounds made it possible to obtain a plot for K_R versus molecular weight which extended well beyond the values obtained for the largest subunit of the guinea pig protein, assuring that the molecular weights were being obtained from a linear portion of the

curve. The study of Vassart, et al. (20) on newly synthesized beef thyroglobulin subunits suffers from the fact that myosin is the highest molecular weight standard employed, making it impossible to predict the shape of the curve to be extrapolated beyond that point and leading to uncertainty in regard to the molecular weight which they obtained.

The values reported here for the major subunits of the guinea pig thyroglobulin do not provide a simple explanation of the overall construction of this protein. The size of the largest subunit obtained is greater than that of a quarter-molecule, but smaller than that of a half-molecule. The possibility that three chains comprise a molecule of 670,000 is unlikely because of the firm data supporting dissociation of the protein under mild conditions into components 335,000 in molecular weight (17). It has previously been proposed that posttranslational proteolysis of a calf thyroglobulin precursor larger than 670,000 produces the nonstoichiometric aminoterminal residues observed as well as the subunit heterogeneity (1). A cleavage of guinea pig thyroglobulin concomitant with iodination has also been suggested (21). It seems likely from the data presented here that the subunits obtained, both major and minor components (Fig. 2), represent cleavage products, either from a "prothyroglobulin" or of biosynthetic subunits initially the size of the half molecule.

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