Thyroglobulin Structure-Function

ISOLATION AND CHARACTERIZATION OF A THYROXINE-CONTAINING POLYPEPTIDE FROM BOVINE THYROGLOBULIN

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Upon reduction and alkylation, 19 S bovine thyroglobulin gave rise to a family of polypeptides ranging in molecular weight from 10,000 to 330,000. This group of polypeptides has been fractionated by chromatography on agarose gel in 6 M urea. After observing significantly increased iodine and thyroxine contents in the lowest molecular weight fraction obtained from the agarose gel-6 M urea chromatography, subsequent fractionation led to the isolation of a single, iodine-rich polypeptide in homogeneous form. While several low molecular weight polypeptides were found to contain iodine, one peptide, designated TG-F, was distinguished by the fact that its iodine content exceeded 2% and virtually all of the iodine found within its structure was in the form of thyroxine. TG-F was purified from pooled normal bovine thyroid tissue with an average yield of 0.9 mol of polypeptide/mol of 19 S thyroglobulin. It contains 2% iodine, only traces of carbohydrates, and 0.4 mol of thyroxine/mol of peptide. The molecular weight of the polypeptide was found to be 10,300 ± 1,300 as determined from sodium dodecyl sulfate gel electrophoresis, thin layer gel filtration in 6 M guanidine hydrochloride, and gel filtration on a calibrated column in ammonium bicarbonate. TG-F contains no free NH$_2$-terminal acid as judged from automated sequenator analysis as well as the dansyl-amino acid procedure. Amino acid analysis revealed 2 lysines, 7 arginines, and a single methionine/mol of peptide. Sodium dodecyl sulfate polyacrylamide gels following cyanogen bromide digestion contained two peptide fragments of similar size and near half the size of TG-F. The absence of significant amounts of iodinated tyrosine derivatives except thyroxine in TG-F suggests that this peptide contains a specific site at which thyroxine formation proceeds both immediately and to completion following iodination of this region by thyroid peroxidase.

Thyroglobulin, the iodinated glycoprotein of the thyroid gland, forms the matrix within which thyroid hormones are synthesized. While much is known about this protein (1-4), structure-function studies have been difficult due to its very large size as well as the physical and chemical microheterogeneity described by a number of workers (5-7). Newly synthesized thyroglobulin is a substrate for thyroid peroxidase, a membrane-associated glycoprotein (8), which catalyzes both iodination and subsequent coupling of iodinated tyrosines to form thyroid hormones (9, 10).

The nature of the thyroid peroxidase reactions has been studied in several laboratories (9, 10), and while significant questions remain to be answered with regard to the mechanism of this enzyme, it has been reported that those tyrosine residues in thyroglobulin which are iodinated early are also those residues which participate in hormone formation (11) and that the efficient formation of hormone occurs over a relatively narrow range of iodine content (12). While the interesting recent report of Cahnman (13), describing small peptides capable of thyroid hormone synthesis, suggests that a specific juxtaposition of 2 tyrosines in a polypeptide structure is important in thyroxine biosynthesis, the nature of the amino acid sequence in the thyroglobulin molecule which produces thyroid hormone has not been defined. Although an initial attempt by Dunn (14) to address this question has provided some data derived from thyroxine-containing small peptides isolated from a pronase digest of rabbit thyroglobulin, only the immediate neighbors to thyroxine within the amino sequence of thyroglobulin were suggested and the specific amino acid sequence surrounding the site of hormone formation remains to be defined.

While there are in excess of 100 tyrosine residues/mol of thyroglobulin in those vertebrate species which have been studied (15, 16), less than a quarter of those tyrosines are normally iodinated in vivo and a still smaller number of such iodinated tyrosines (typically between 4 and 8) are actually involved in hormone synthesis. That very specific tyrosines are involved in hormonogenesis is further suggested by the unique ability of thyroglobulins to form thyroid hormone at very low levels of iodination (17) and by the recent observation of Dunn (18) that specific peptide fragments derived from rabbit thyroglobulin are involved in thyroxine biosynthesis.

While thyroid hormone synthesis has been reported in several other proteins at high levels of iodination (17), the thyroglobulins alone, among the many proteins studied, are capable of significant thyroid hormone synthesis at low levels of iodination.

In this paper, we report the isolation and partial characterization of a polypeptide fragment, derived from normally iodinated bovine thyroglobulin upon reduction and alkylation. This peptide contains significant amounts of thyroxine and no significant amounts of other iodoamino acids. This polypeptide fragment contains an amino acid sequence which is clearly important in hormone formation since it contains one-third of the total thyroxine in the native thyroglobulin. Moreover, it is of sufficiently small size to permit amino acid sequence studies.

**EXPERIMENTAL PROCEDURES**

Preparation of Thyroglobulin—Bovine thyroid glands were ob-
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tained at a local slaughter house from healthy cattle. The thyroids were removed and kept on ice until use (within 30 min) or frozen within 10 min after the killing of the animals in the case of later use. The bovine thyroids were sectioned into 1- to 2-mm wide slices with a single-edged razor, and extracted overnight at 4 °C in 400 ml of 0.15 M NaCl, pH 7.5/100 g of tissue. When frozen thyroids were used, they were thawed slowly to avoid freezing injury (see figure legends). Dansyl amino acid analysis was carried out on a Gilford spectrophotometer equipped with a gel-scanning apparatus.

NH₂-Terminal Group Determination—Samples of purified TG-F peptide from the P-60 column were subjected to NH₂-terminal analysis of the dansyl amino acid technique of Woods and Wang (30). The peptide was also subjected to automated analysis (33) in a Beckman model 890C sequenator (using Beckman Program 102974). The fractions obtained from the sequenator were subjected to both high performance liquid chromatography analyses (34) and back-hydrolysis followed by amino acid analysis in order to identify released amino acids (35).

Pyroglutamic Acid Aminopeptidase Digestion—TG-F was treated with pyroglutamyl aminopeptidase according to the method of Podell and Abraham (36). Molecular Weight Determinations—The molecular weights of the reduced and alkylated peptides were determined in SDS-gel electrophoresis (37), migration in the thin layer gel filtration system of Hung et al. (38) in 6 m guanidine HCl, and from elution behavior on a calibrated column of Bio-Gel P-60 (Bio-Rad) (39). In each case, standards consisted of reduced and alkylated proteins or polypeptides of known molecular weight, as indicated in the figure legends. High Performance Liquid Chromatography Analyses—High performance liquid chromatography of reduced and alkylated peptides was carried out on a Beckman-Altex model 332 liquid chromatograph using a Waters I-123 protein column in 0.1% ammonium bicarbonate. Column effluent was monitored at 230 nm.

Tryptic Digestion and Peptide Mapping—Tryptic digests were carried out as previously described (40) in 1% ammonium bicarbonate. Aliquots of the tryptic digest were spotted on a sheet (20 × 20 cm) of thin layer cellulose (Eastman-Kodak (without fluorescent indicator)) and chromatographed in an ascending chamber in butanol:pyridine:acetic acid:water (5:10:2:12). After drying, the plates were sprayed with a dilute pyridine:acetic acid buffer at pH 3.5 and electrophoresed in the second dimension. Tryptic peptides were detected with a spray of 0.1% ninhydrin in ethanol or with fluorescamine and ultraviolet light. Iodine-containing peptides were detected with the Gennil and Vironen reagent (41) in the form of a fine spray. Iodine-containing peptides were revealed initially as dark blue spots against a yellow-green background. Substantial background deterioration occurred with time, necessitating observation and recording of the iodine staining within 5 to 10 min.

Cyanoazobromide Cleavage—TG-F was subjected to CNBr cleavage in 70% formic acid (42). After removal of the formic acid and CNBr, the sample was concentrated by rotary evaporation and an aliquot applied to an SDS-polyacrylamide gel for analysis of cleavage products.

Estimation of Stoichiometry—The stoichiometry of TG-F per mol of reduced and alkylated thyroglobulin was determined by densitometry of 4% SDS-polyacrylamide gels. Coomassie blue staining was assumed to be proportional to the amount of each polypeptide component. The amount of TG-F recovered from the CL-4B column chromatography was determined by integration of the absorbance at 280 nm. An E₂ｅ₅₀ of 15.8 based on amino acid analyses and Lowry determinations on pure TG-F was used in the latter determination.

RESULTS

19 S Thyroglobulin Starting Material—The 19 S thyroglobulin used in these studies was isolated from pooled bovine thyroids following ammonium sulfate fractionation and 4% agarose gel chromatography. The fractions pooled from such a column yield a preparation which is over 95% in the 19 S form when subjected to direct analytical ultracentrifugation before freeze-drying. The remaining components in the preparation, the 12 S and 27 S forms of thyroglobulin, are present in slightly increased amounts following lyophilization due to dissociation but do not exceed 15% of the total sedimenting material. Gel electrophoresis of the material showed the usual three-banded pattern seen in thyroglobulins (15). The iodine

1 The abbreviations used are: 19 S thyroglobulin, thyroglobulin which sediments in the ultracentrifuge with a sedimentation coefficient near 19 S; SDS, sodium dodecyl sulfate; TG-F, the lowest molecular weight polypeptide derived from reduced and alkylated bovine thyroglobulin; dansyl, 5-dimethylaminonaphthalene-1-sulfonfyl. 
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Fractionation of Reduced and Alkylated Thyroglobulin—
Sodium dodecyl sulfate electrophoresis of reduced and alkylated thyroglobulin revealed a family of polypeptides ranging in size from $M_r$ approximately 10,000 to near 330,000. No significant difference was observed between the SDS-gel electrophoresis patterns of the thyroglobulin prepared from fresh tissues and that prepared from frozen glands. Therefore, in most cases, the thyroglobulin was prepared from frozen glands. This mixture of reduced and alkylated polypeptide chains was applied to a column of Sepharose CL-4B in 6 M urea, pH 7.0, 0.1 M sodium phosphate (Fig. 1). The elution profile revealed a large initial peak of high molecular weight material with several shoulders on the low molecular weight side followed by four additional peaks at lower molecular size. These fractions were designated A through F in order of decreasing molecular size (Fig. 1). After dialysis against 0.1% NH$_4$HCO$_3$, each pooled fraction was freeze-dried. The fractions were each analyzed for iodine content (see Table I) and were subjected to SDS-gel electrophoresis (Fig. 2). Due to their iodine content and our earlier observations (45), we focused our attention on the lower molecular weight fractions. Fractions E and F were found to contain increased amounts of iodine (1.6% and 2.1%, respectively) when compared to the starting protein (15). While fraction D was complex and contained a number of bands of intermediate molecular size, fraction E contained two principal components with molecular weights near 20,000 and a small amount of a third component which corresponded to the principal constituent of fraction F. Fraction F contained reduced amounts of the two components seen in fraction E and a principal constituent of $M_r$ near 10,000. Analyses of fractions E and F for thyroxine indicated that fraction F, the lowest molecular weight fraction, contained a substantial amount of thyroid hormone. Moreover, the per cent of the total iodine present in this form in this fraction was very high (>90%) compared to that of the starting protein (15%).

The material in fraction F of the CL-4B Sepharose column was next applied to a column of Bio-Gel P-60 in an attempt to further purify the principal component in this fraction. The results of such an experiment are shown in Fig. 3. Three peaks were seen in the elution profile with the major peak representing material which corresponded to the principal component seen in gel electrophoresis of the impure fraction F from the urea-CL-4B column. The two lesser peaks seen in the elution profile corresponded with the two principal components seen in fraction E of the urea-CL-4B column. These components were designated TG-E-1, TG-E-2, and TG-F, in order of their elution.

Characterization of the Purified Low Molecular Weight Iodinated Peptides—Peptides TG-E-1, TG-E-2, and TG-F were shown to be enriched in both iodine and thyroxine (see Table II). Most striking was TG-F, which not only showed an

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**Table I**

<table>
<thead>
<tr>
<th>Tg fraction</th>
<th>Iodine</th>
</tr>
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<tbody>
<tr>
<td>19 S TG</td>
<td>1.0</td>
</tr>
<tr>
<td>TG-A</td>
<td>0.97</td>
</tr>
<tr>
<td>TG-B</td>
<td>1.1</td>
</tr>
<tr>
<td>TG-C</td>
<td>0.95</td>
</tr>
<tr>
<td>TG-D</td>
<td>0.88</td>
</tr>
<tr>
<td>TG-E</td>
<td>1.6</td>
</tr>
<tr>
<td>TG-F</td>
<td>2.1</td>
</tr>
</tbody>
</table>

$^a$ Tg = thyroglobulin.
increased iodine content, but virtually all of its iodine was in the form of thyroxine as determined by specific radioimmunoassay. Because of its unique properties and composition, specific attention was directed to the characterization of TG-F.

The Properties of TG-F—SDS-polyacrylamide gel electrophoresis of TG-F revealed a single band migrating at a rate consistent with a molecular weight of near 10,000 (Fig. 4). The data obtained using three different techniques for the determination of molecular weight are summarized in Fig. 5. TG-F showed an apparent molecular weight of 10,300 ± 1,300 and was seen as a single component in all three procedures.

The results of amino acid, iodoamino acid, and carbohydrate analyses of TG-F are summarized in Table III. TG-F contains a single methionine residue, 2 lysine, and 7 arginine residues. The small amounts of neutral sugar and glucosamine found in the peptide do not correspond to any of the known oligosaccharide units in bovine thyroglobulin (43). Since the carbohydrate-staining procedure of Fairbanks (31) was negative for gels containing TG-F, definition of significance of the neutral sugars found in the analyses must await further characterization of the peptide. The amino acid and carbohydrate compositions shown in Table III are consistent with a molecular weight of approximately 9,800. This compares very well with the value obtained in physical studies, 10,300 ± 1,300.

Treatment of TG-F with CNBr followed by SDS-polyacrylamide gel electrophoresis resulted in two bands after Coomassie blue staining with similar molecular sizes near half that of TG-F, suggesting that the single methionine found in the peptide occurs near the middle of the polypeptide chain.

Two-dimensional peptide maps of a tryptic digest of TG-F contained 11 spots when sprayed with ninhydrin or fluorescamine. Spraying of such a tryptic peptide map of TG-F with the iodine spray of Gmelin and Virtanen (41) revealed two rapidly developing positive spots.

When TG-F was subjected to 10 cycles of automated Edman degradation in a sequenator, no free NH$_2$-terminal sequence could be found either by chromatography of phenylthiohydantoin amino acid derivatives or back-hydrolysis followed by amino acid analyses. When this observation was confirmed in a repeat experiment at higher sample load, dansylation of TG-F followed by hydrolysis and thin layer chromatography with standard dansyl amino acid markers was carried out and revealed no spot which corresponded to a known a-dansylated amino acid.

Since Hayashi et al. (44) reported that pyroglutamic acid is a blocking group at the NH$_2$-terminus of hog thyroglobulin, TG-F was treated with pyroglutamic acid aminopeptidase (36) and then resubmitted to the automated Edman procedure. While negative results were obtained again, pyroglutamate

| TABLE II |
| Thyroxine content of the purified small molecular weight fractions of 19 S thyroglobulin |

<table>
<thead>
<tr>
<th>Tg fraction</th>
<th>Iodine</th>
<th>Amount T4/mol peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_1$</td>
<td>0.95</td>
<td>0.1</td>
</tr>
<tr>
<td>E$_2$</td>
<td>1.7</td>
<td>0.2</td>
</tr>
<tr>
<td>F</td>
<td>2.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Fig. 4. Ten % sodium dodecyl sulfate polyacrylamide tube gel electrophoresis of TG-F. The upper portion shows the gel; the lower portion shows the densitometer scan at 595 nm. Sample size was 40 μg.*

*Fig. 5. Calibration curves for the three methods used to determine the molecular weight of TG-F. The following reduced and alkylated standards were used with numbers corresponding to those in the figure: 1) bovine serum albumin; 2) ovalbumin; 3) chymotrypsinogen; 4) horse myoglobin; 5) α-lactalbumin; 6) ribonuclease; 7) cytochrome c; 8) horse myoglobin CNBr fragment (M, 8,000); and 9) horse myoglobin CNBr fragment (M, 6,000). The arrows represent the position of TG-F in each case. Panel A shows the data from a calibrated P-60 column in 0.1% NH$_4$HCO$_3$. Panel B shows the results of thin layer gel chromatography in G-75, in 6 M guanidine HCl. Panel C shows the data from sodium dodecyl sulfate polyacrylamide gel electrophoresis in 10% gels.*

| TABLE III |
| Amino acid and carbohydrate composition |

<table>
<thead>
<tr>
<th>TG-F</th>
<th>19 S Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>mol/mol peptide</td>
<td>% wt*</td>
</tr>
<tr>
<td>Carboxymethylcysteine</td>
<td>3.22</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.00</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.12</td>
</tr>
<tr>
<td>Serine</td>
<td>5.97</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>14.19</td>
</tr>
<tr>
<td>Proline</td>
<td>6.25</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.02</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.69</td>
</tr>
<tr>
<td>Valine</td>
<td>4.74</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.57</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.78</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.30</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.41</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.08</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.14</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.15</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.47</td>
</tr>
<tr>
<td>Tryptophan*</td>
<td>1.90</td>
</tr>
<tr>
<td>N-Acetylgalcosamine</td>
<td>0.98</td>
</tr>
<tr>
<td>Fucose</td>
<td>0.74</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.78</td>
</tr>
<tr>
<td>Galactose</td>
<td>1.15</td>
</tr>
<tr>
<td>Monoiodotyrosine</td>
<td>0.237'</td>
</tr>
<tr>
<td>Diiodotyrosine</td>
<td>0.518'</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>0.026'</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Average of five analyses.

* These data are taken from Gleason (28).

* Determined by assuming 8.00 mol of aspartic acid/mol of TG-F.

* Per cent of weight recovered.

* Determined spectrophotometrically.

* Per cent recovered from ion exchange column.
acrylamide gel electrophoresis experiments. E; must be regarded as a representative yield of TG-F from since the larger molecular weight fractions may contain ad-

These observations suggest that very near 2 mol of TG-F
occurred/mol of thyroglobulin. TG-F is recovered in amounts of

Based on integration of the area under the TG-F peak and an
% of 15.8, determined from purified TG-F.

S. Kim, and D. Dunn at the annual
meeting of The American Thyroid Association, San Diego, CA, No-

Discussion
In 1975, we reported that the smallest polypeptide frag-
ments observed upon reduction and alklylation of bovine thy-
roglobulin were specifically enriched in both iodine and thy-
roid hormone (45). That observation has been recently con-
firmed and extended by Dunn et al. (18) in rabbit and human
thyroglobulins. In this paper, we describe the isolation of one
such small peptide (TG-F) derived from bovine thyroglobulin,
and/or diiodotyrosine in TG-F is in stark contrast to the
iodoamino acid composition of whole thyroglobulin and other
iodinated peptides where substantial amounts of both of these
iodoamino acids are found. This difference suggests that when
the tyrosine residues in TG-F are iodinated, subsequent
coupling to form thyroxine proceeds immediately and com-
pletely. While these data may be interpreted in other ways,
this suggestion is consistent with the fact that thyroid perox-

The presence of significant amounts of monoiiodotyrosine
and/or diiodotyrosine in TG-F is not particularly unusual, although the presence of a single methionine near
the middle of the TG-F sequence is notable in that the CNBr
cleavage products should be useful in providing overlaps for
amino acid sequence studies. The number of tryptic peptides
observed in peptide maps (10–12) is consistent with the 2
lysine and 7 arginine residues found. The absence of histidine
in TG-F should also be noted. It is of interest that the tyrosine
content in TG-F is significantly lower than that of thyroglob-
ulin. Moreover, when one assumes that both of the thyroasines
which form the thyroxine come from the TG-F sequence, the
remaining 1.4 residues of noniodinated tyrosine found in TG-
F would be totally consumed in hormone formation if the
peptide were fully iodinated and coupled (to a level of 1
thyroxine/mol of TG-F). Even if only half of the thyroxines in
TG-F were iodinated, the fully iodinated and coupled peptide would contain only a single remaining tyro-
sine residue not used during hormone formation. This obser-
vation is quite consistent with the concept of very specific
sites on bovine thyroglobulin for hormonogenesis since, of
approximately 120 tyrosine residues found in thyroglobulin,
only 25 to 30 are iodinated and less than 6 (~5%) participate
in thyroxine formation.

While small amounts of carbohydrate were found upon
analysis of TG-F, the relative and absolute amounts of sugars
do not correspond to any of the known oligosaccharide units
reported in bovine thyroglobulin, and it is possible that these
small amounts of carbohydrate reflect either a new oligosac-
charide type in bovine thyroglobulin or possibly some minor
contamination of the preparation with a glycosylated compo-
nent(s). The fact that staining of polyacrylamide electropho-
resis gels containing TG-F with the Schiff-periodate stain
according to the method of Fairbanks et al. (30) was negative
suggests that the peptide may, in fact, be nonglycosylated.

The absence of a free NH₂ terminus in TG-F is significant
in view of the fact that in multiple studies by several groups
(15, 46, 47), only limited amounts of NH₂-terminal amino acids
have been reported (insufficient amounts to be consistent with
the multiple polypeptide fragments observed in almost every

**Table IV**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Amount TG-F recovered/100 mg reduced and alkylated thyroglobulin</th>
<th>Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction and alkylation</td>
<td>3.1*</td>
<td>100</td>
</tr>
<tr>
<td>CL-4B chromatography</td>
<td>2.9c</td>
<td>94</td>
</tr>
<tr>
<td>P-60 chromatography</td>
<td>1.4</td>
<td>45</td>
</tr>
</tbody>
</table>

* These are representative values obtained from over 20 individual samples of reduced and alkylated thyroglobulin.

**Based on densitometry of Coomassie Blue stained 4% SDS-poly-
acrylamide gel electrophoresis experiments.

Based on integration of the area under the TG-F peak and an
% of 15.8, determined from purified TG-F.

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thyroglobulin preparation studied upon reduction).

The origin of the TG-F polypeptide is an important question which is currently being addressed in this laboratory. Since it is found in disulfide linkage with the 19 S thyroglobulin complex, the simplest assumption is that it is derived from a parent polypeptide chain of thyroglobulin by either proteolysis or some nonclassical peptide bond cleavage. Whether this cleavage is part of the normal processing of thyroglobulin polypeptide chains and whether the cleavage proceeds or follows iodination and coupling are questions which remain to be answered. On the other hand, the possibility of independent synthesis of TG-F followed by disulfide linkage to the thyroglobulin complex cannot be ruled out at this time, particularly in view of the recent observations by Dunn and co-workers (48, 49) that thyroglobulin from different thyroids within the same species and even from different parts of a single gland may have different amino acid compositions and presumably different amino acid sequences. If the observations of Dunn and co-workers are confirmed, clearly, the generally accepted scheme for thyroglobulin biosynthesis as unique chains of M, 330,000 (50) must be re-examined.

Regardless of its origin, TG-F represents a fragment of the thyroglobulin complex which contains an amino acid sequence of key significance in thyroid hormone biosynthesis and thyroglobulin structure-function and therefore merits further study including the determination of the sequence and the location of the hormonogenic site within that sequence.

It should be noted that recent reports from Dunn and co-workers (18) have described the isolation of a similar peptide derived from reduced and alkylated rabbit thyroglobulin following in vitro iodination of the protein with radioidine. The polypeptide fragment described by Dunn et al. differs in several respects from TG-F. It has a reported molecular size of 20,000 (twice the size of TG-F), contains 10% carbohydrate and a free NH₂-terminal aspartic acid. Moreover, the rabbit peptide contains significant amounts of other iodoamino acids in addition to thyroxine. It will be of interest to compare amino acid sequence data on these two hormonogenic peptides as they become available.

Acknowledgments—We would like to thank Linda Fox for able technical assistance. The contribution of Dr. M. J. Gleason to the early stages of this work is also gratefully acknowledged. We would also like to thank Dr. A. Taurog at The University of Texas Center for the Health Sciences, Dallas, for carrying out the thyroxine radioimmunoassays and Dr. John Dunn at The University of Virginia School of Medicine, Charlottesville, for allowing us to see a copy of amino acid sequence data on these two hormonogenic peptides.

REFERENCES