Favored Sites for Thyroid Hormone Formation on the Peptide Chains of Human Thyroglobulin*

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Gel electrophoresis of reduced thyroglobulin from normal human thyroids showed five major iodinated components, with estimated molecular masses of >300,000 daltons (14% of thyroglobulin’s iodine), 230,000 (30%), 45,000 (4.5%), 26,000 (17%), and 18,000 (4%). The 26,000- and 18,000-dalton components had over 50% of their iodine as thyroxine. The 26,000-dalton iodopeptide had a single NH2-terminal, contained 29% of thyroglobulin’s thyroxine, and was present in a ratio of approximately 1 mol/mole of 19 S thyroglobulin.

In vitro addition of iodine to low iodine thyroglobulin, in increments of 0 to 120 mol of elemental iodine/mole of protein, showed the 230,000- and 45,000-dalton components to be iodinated preferentially, followed by the 26,000-dalton component with lower iodine content. Iodination on the 230,000- and 26,000-dalton components produced iodotyrosines, while that on the 26,000- and 18,000-dalton components had over 50% of their iodine as thyroxine. The 26,000-dalton iodopeptide had a single NH2-terminal, contained 29% of thyroglobulin’s thyroxine, and was present in a ratio of approximately 1 mol/mole of 19 S thyroglobulin.

We conclude: 1) the 26,000-dalton iodopeptide contains the most favored site for thyroid hormone synthesis; 2) iodotyrosine formation first occurs on the 230,000-dalton component, and this iodotyrosyl is a probable precursor to the iodothyronine of the 26,000-dalton iodopeptide. Pulse-labeling experiments with iodination of low iodine thyroglobulin suggested a transfer of iodine from the 230,000-dalton component to the 26,000-dalton one, and further transfer from these or other precursors to the 18,000- and >300,000-dalton component at high iodine levels.

Many proteins can form iodotyrosyl residues in vitro, but thyroglobulin is much more proficient than others in forming iodothyronines (1). In nature, the thyroid hormones, thyroxine and 3,5,3'-triiodothyronine, occur only in thyroglobulin. The proposed mechanism for thyroxine biosynthesis is by diphenyl ether linkage between two diiodotyrosyls from different regions of the thyroglobulin molecule, with loss of the alanine side chain from the outer diiodophenyl ring (1). Details of this reaction and of thyroglobulin’s part in it are lacking. In a recent report (2), we found that rabbit thyroglobulin contained discrete iodopeptides of small molecular weight with a high thyroxine content. The present work shows that human thyroglobulin also has thyroxine-rich iodopeptides of low molecular weight, and describes how varying levels of iodine influence iodothyronine formation in different parts of the molecule.

EXPERIMENTAL PROCEDURES

Thyroglobulin Samples

Preparation I—We obtained 49 g of thyroid tissue from autopsies of nine individuals without evidence of thyroid disease. The thyroid were sliced, homogenized at 2 °C in a buffer of 0.05 M sodium phosphate, pH 7, which contained the proteolytic enzyme inhibitors pepstatin (10−5 M) and phenylmethanesulfonyl fluoride (10−5 M), and centrifuged. Thyroglobulin was isolated from the supernatant on a column (5 × 100 cm) of Bio-Gel A-5M at 4 °C in 0.05% sodium phosphate, pH 7, made 0.02% with NaN3, as previously described (2).

Preparation II—Thyroid tissue was obtained at surgery from a patient undergoing thyroidectomy for goiter, and transported on ice immediately to the laboratory where it was placed in phosphate buffer with pepstatin and phenylmethanesulfonyl fluoride, sliced, homogenized, and fractionated by gel filtration as above. This thyroglobulin had an iodine content of 5 ng/μg of protein.

Preparation III—Thyroglobulin was purified by gel filtration from the large goiter of a patient with Pendred’s Syndrome, as described elsewhere (preparation CS-I in Ref. 3). Its iodine content was 0.5 ng/μg of protein. Unless otherwise stated, this preparation was used for all in vitro iodination experiments.

Other Preparations—Details of the purification of thyroglobulin from several normal and goitrous thyroids have been published (4). Other samples were from patients with nontoxic goiters removed at surgery, with thyroglobulins being isolated by gel filtration. The purity of each thyroglobulin sample was assessed by electrophoresis on 4% gels at pH 8.9 without reduction. Each of these, as well as preparations I, II, and III showed only the two- or three-handed pattern typically associated with purified thyroglobulin, as detailed in numerous publications (e.g. 4–7).

Iodination of Thyroglobulin in Vitro

We followed the general method of Taurog et al. (8) for continuous iodination. Each milliliter of incubation mixture, in 25 mM sodium phosphate, pH 6.7, included: thyroglobulin, 1 mmol (M, 660,000); glucose, 0.9 mg; glucose oxidase (EC 1.1.3.4 (Sigma)), 5 μg; lactoperoxidase (EC 1.11.1.7 (Sigma)), 5 μg; sodium-125I, carrier-free, approximately 2.5 μCi; and KI in varying amounts from 0 to 200 μmol. Routine incubations were at 37 °C for 1 h. Samples with the lactoperoxidase omitted were run as controls. The fraction of added 125I incorporated into protein was assessed for all samples by paper chromatography, and showed label only in iodide and at the origin, with no evidence for the production of free thyroxine or other iodoamino acids during iodination. Following incubation, we made the...
samples 2% mercaptoethanol and 1% SDS 1 placed them for 5 min in
boiling water, and applied them to gels in SDS for electrophoresis.

For pulse labeling with iodine, we first iodinated with carrier-free
sodium 125I, plus fixed amounts of KI if desired, then dialyzed against
water overnight to remove unreacted 125I, then conducted a second
iodination in new incubation medium with KI at fixed amounts and
123I added.

For all iodination experiments, the atoms of iodine introduced in
vitro need to be added to those already in thyroglobulin to obtain the
final iodine content. For preparation III, there were 2.6 atoms of
iodine already present, so addition of e.g. 20 atoms in vitro would
give 23 atoms total.

Other Techniques

We performed the following procedures as previously described (2):
(a) gel electrophoresis in SDS, followed by slicing into horizontal
segments for 123I counting, for 127I determinations, or for elution, (b)
reduction of thyroglobulin with mercaptoethanol and alklylation with
acrylamide or iodoacetic acid; (c) separation of iodinated compo-
nents of reduced thyroglobulin on Bio-Gel A-5M, DEAE-cellulose,
and preparative polyacrylamide gel electrophoresis; (d) determination
of labeled iodide and iodoamino acids, after digestion with promase,
by paper chromatography in butanol:ethanol:1 N HNO3 (5:1:2, v/v/v),
butanol:2 N acetic acid, or 2% amyl alcohol saturated with 2 N
HNO3; (e) thyroxine by radioimmunoassay (9); (f) NH2 terminals
by reaction with dansyl chloride and two-dimensional thin layer
chromatography; (g) iodine by ceric sulfate reduction after digestion
with chloric acid; (h) amino acid analyses on a Dionex automated
 analyzer with integrator, following digestion of peptide samples with
cysteine solution in constant boiling HCl in evacuated tubes at
105 °C for 3 h. These conditions gave suitable recovery in our previous work with rabbit
thyroglobulin (6).

RESULTS

Iodopeptides of Thyroglobulin

Fig. 1 shows the distribution of iodine on gel electrophoresis of
reduced human thyroglobulin from preparation I. Iodogen
was consistently found in five bands or zones, labeled A to E in
Fig. 1, with approximate molecular masses of >300,000,
230,000, 45,000, 26,000, and 18,000 daltons, respectively, as
estimated from markers on parallel gels. Variable amounts of
iodinated peptide material were also found in zones between
60,000 and 200,000 daltons (from 30 to 50 mm in the gel
diagram in Fig. 1); these were not studied further, since
cfractions in this size range invariably have a low iodothyronine
content in studies with isotopically labeled thyroglobulin,
and they are difficult to isolate in bulk. The three smaller
iodopeptides (C, D, and E of Fig. 1) were first separated from
the larger components by gel filtration of reduced and alkylated
thyroglobulin on Bio-Gel A-5M in 0.1% SDS, and then sepa-
rated from each other by preparative electrophoresis on gels of
8% polyacrylamide. With the 26,000- and 18,000-dalton
iodopeptides, we interopolated an additional isolation step on
DEAE-cellulose with a discontinuous gradient of increasing
concentrations of NH4HCO3 in 6 M urea. The individual
iodopeptide fractions were cluted from the preparative gel,
then dialyzed, and lyophilized. These purification procedures
followed closely those previously described for small iodopep-
tides from rabbit thyroglobulins (2). The heavier components
(A and B of Fig. 1) emerged from the A-5M column, respec-
tively, at the leading and trailing edges of a single broad peak.
Each could be located by its pattern on analytical gel electro-
phoresis, and fractions containing it were pooled accordingly,
followed by dialysis and lyophilization. Each of the five puri-
fied components (A to E of Fig. 1) gave a single stained band
after analytical gel electrophoresis in SDS.

Table I shows the distribution of iodine and thyroxine in
thyroglobulin and among these five iodopeptides, and Table

1 The abbreviations used are: SDS, sodium dodecyl sulfate; dansyl,
5-dimethylaminonaphthalene-1-sulfonyl.

Fig. 1. Iodine distribution on electrophoresis of reduced hu-
man thyroglobulin (preparation I) on gels of 4% polyacryl-
amide in 0.1% SDS. Ordinate is 125I content in nanograms per 2.5-
mm gel segment; abscissa shows location on gel, with direction
of migration to right.

Table II gives its relative distribution of amino acids. Calculations
of approximate protein content were based on the amino acid
analysis. Since sufficient amounts of purified material were not
available for direct determination of carbohydrate or
thyroxine, we assumed values of 10% for carbohydrate and
1.8% for thyroxine, based on our previous data for normal
human thyroglobulin (4). We measured the 125I content of
each aliquot taken for protein and thyroxine analysis, and
used it to inter-relate protein, thyroxine, and the fraction of
thyroglobulin's iodine on gel. Sample calculations are shown
in the footnotes of Table I. Both the 26,000- (component "D")
and 18,000- (component "E")-dalton iodopeptides had iodine/protein
ratios several times greater than those of thyroglobulin
and the three heavier iodinated components, and both had
most of their iodine in the form of thyroxine. Together, the
26,000- and 18,000-dalton iodopeptides accounted for 35% of
thyroglobulin's thyroxine but only 6% of its protein. The five
components listed in Table I accounted for 69% of the iodine
of thyroglobulin, 67% of its protein, and 85% of its thyroxine.

The molar ratio of each iodinated component to thyroglobulin
(660,000 daltons) was calculated from its amino acid
content, its iodine content in 5-cyanomethylated preparations,
the fraction of thyroglobulin's iodine which it contained, and
its molecular weight as estimated from gels. These calculated
values are approximate, since they include assumptions about
iodine and tryptophan content, and rely on gels for
molecular weight estimates. They show that each mole of
thyroglobulin contained approximately 1 and of 26,000-dalton,
between 1 and 2 mol of 230,000-dalton, and less than 1 mol
each of 45,000-, 18,000-, and >300,000-dalton iodopeptides
(Table I).

Table II shows that the >300,000- and 230,000-dalton
iodopeptides were virtually identical with thyroglobulin in
amino acid distribution, while each of the three smaller com-
ponents differed from the heavier components and from each
other. The 26,000-dalton iodopeptide had a single NH2 ter-
minal, aspartic acid. There was not sufficient material to
determine NH2 terminals on 45,000- and 18,000-dalton iodo-
peptides.

Preparation II was isolated with minimal opportunity for postmor
tem proteolysis. It showed an iodine distribution similar
to that of Fig. 1, with well defined peaks at 45,000 daltons
(5.5% of total iodine on gel), 26,000 daltons (9.7%), and 18,000
daltons (4.8%).

In Vitro Iodination

Continuous Labeling—Goiter thyroglobulin (preparation

1.9% for tryptophan, based on our previous data for normal
human thyroglobulin (4). We measured the 125I content of
each aliquot taken for protein and thyroxine analysis, and
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to that of Fig. 1, with well defined peaks at 45,000 daltons
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daltons (4.8%).
Favored Sites for Thyroxine Formation

**TABLE I**

<table>
<thead>
<tr>
<th>Human thyroglobulin and its major iodinated components</th>
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</thead>
<tbody>
<tr>
<td><strong>Thyroglobulin</strong></td>
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<tr>
<td>Approximate molecular mass (kilodaltons)</td>
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<tr>
<td>Iodine content (mg I/g protein)</td>
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<tr>
<td>Share of thyroglobulin’s iodine (%)</td>
</tr>
<tr>
<td>Fraction of iodine as thyroxine (%)</td>
</tr>
<tr>
<td>Share of thyroglobulin’s thyroxine (%)</td>
</tr>
<tr>
<td>Residues of thyroxine/mol thyroglobulin</td>
</tr>
<tr>
<td>Moles of component/mol thyroglobulin</td>
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<table>
<thead>
<tr>
<th><strong>Iodinated component</strong></th>
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<tr>
<td>A</td>
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<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>Share of thyroglobulin’s iodine (%)</td>
</tr>
<tr>
<td>3.5</td>
</tr>
<tr>
<td>3.5</td>
</tr>
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</tr>
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<td>23.8</td>
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<td>14.5</td>
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<tr>
<td>Fraction of component’s iodine as thyroxine (%)</td>
</tr>
<tr>
<td>0.82</td>
</tr>
<tr>
<td>0.16</td>
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<td>0.55</td>
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</table>

*Letters refer to components shown in Fig. 1. The text describes their purification.

Calculated as (fraction of thyroglobulin’s iodine in component) times (fraction of component’s iodine as thyroxine divided by fraction of thyroglobulin’s iodine as thyroxine) times (residues of thyroxine in each mole of thyroglobulin), e.g. for 26,000 daltons, 0.169 × 0.60/0.35 × 2.82 = 0.82.

Calculated as (fraction of thyroglobulin’s iodine in component) times (iodine content of thyroglobulin divided by iodine content of component) times (M₀ of thyroglobulin divided by M₀ of component), e.g. for 26,000 daltons, 0.169 × 0.6/23.8 × 600,000/26,000 = 1.12.

**TABLE II**

<table>
<thead>
<tr>
<th>Amino acid composition of thyroglobulin and its major iodinated components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data expressed as residues per 1000 residues.</td>
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</table>

<table>
<thead>
<tr>
<th>A (≥300,000 daltons)</th>
<th>B (230,000 daltons)</th>
<th>C (45,000 daltons)</th>
<th>D (26,000 daltons)</th>
<th>E (18,000 daltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>81</td>
<td>78</td>
<td>76</td>
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<tr>
<td>Threonine</td>
<td>49</td>
<td>51</td>
<td>50</td>
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<td>Serine</td>
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<tr>
<td>Glutamic acid</td>
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<td>Proline</td>
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<td>68</td>
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<tr>
<td>Glycine</td>
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<td>117</td>
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<td>Alanine</td>
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<td>Half-cystine</td>
<td>31</td>
<td>28</td>
<td>27</td>
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<tr>
<td>Valine</td>
<td>62</td>
<td>57</td>
<td>68</td>
<td>58</td>
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<tr>
<td>Methionine</td>
<td>11</td>
<td>11</td>
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<td>Isoleucine</td>
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<td>Leucine</td>
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<td>Tyrosine</td>
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<td>Phenylalanine</td>
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<td>Histidine</td>
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<td>Lysine</td>
<td>36</td>
<td>34</td>
<td>34</td>
<td>43</td>
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<tr>
<td>Arginine</td>
<td>54</td>
<td>55</td>
<td>55</td>
<td>59</td>
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</table>

*Most values represent the means of determinations on 36-h hydrolysates of S-cyanosethylated samples. Glutamic acid and half-cystine were from S-carboxymethylated samples. No corrections were made for destruction of delayed release during hydrolyses. Total numbers of analyses: thyroglobulin, 7; >300,000-daltons iodopeptide, 3; 230,000-daltons iodopeptide, 4; 45,000-daltons iodopeptide, 2; 26,000-daltons iodopeptide, 11; 18,000-daltons iodopeptide, 4.*

*Letters refer to components shown in Fig. 1.

III) iodinated in vitro showed the same major bands on polyacrylamide gel electrophoresis as did the autopsied-derived material. Fig. 2 shows representative gel patterns, and Fig. 3 quantitates the distribution of ¹³¹I among these bands. Approximate molecular masses were estimated on gels at several different acrylamide concentrations between 6% and 12%. When the ¹³¹I tracer alone was added, most was incorporated into the 230,000-dalton band, with small amounts in 45,000-dalton iodopeptide. With addition of more iodine, less of the total appeared in the 230,000-dalton and more in 26,000-dalton iodopeptide, the latter rising to 31% at 30 atoms of added iodine. At 40 atoms of added iodine, a smaller proportion was found in 26,000-dalton iodopeptide and there were now substantial amounts in the 18,000-dalton band, a trend which continued with still further additions of iodine. It became increasingly difficult to recognize a >300,000-dalton zone with progressive iodination (Fig. 2), and its pattern is omitted in Fig. 3. At 0, 10, and 20 atoms of added iodine it represented, respectively, 5.6, 7.3, and 6.0% of thyroglobulin’s ¹³¹I.

In an additional experiment, we selected four iodination levels, 0, 20, 40, or 60 atoms of iodine/molecule of thyroglobulin (M₀ = 660,000), followed by reduction with mercaptoethanol and electrophoresis in 0.1% SDS. Each diagram is a composite. The portion to the right of the dashed vertical line is from gels of 8% polyacrylamide, and is expressed as the percentage of thyroglobulin’s ¹³¹I found in each gel segment of 2.5 mm. The portion to the left of the dashed vertical line represents an expanded scale of the first 15 mm of the 8% gel; for this, another aliquot of the sample was run on a 3.5% gel; the first 40 mm from the origin, shown to be equivalent to the first 15 mm of the 8% gels by molecular weight markers, was cut into equal segments of 5 mm, and each segment’s percentage of thyroglobulin’s ¹³¹I is shown in the diagrams.

In an additional experiment, we selected four iodination levels, 0, 20, 40, or 60 atoms of added iodine, for studies with a more active ¹³¹I label to permit analysis of iodopeptides eluted from gels. Table III presents the distribution of iodomino acids in thyroglobulin and the major iodinated bands. Only these four iodomino acids had an appreciable content of ¹³¹I. Small amounts at the chromatographic origin and as iodide were attributed, respectively, to undigested material.

**FIG. 2.** Distribution of radioactivity on electrophoretic gels after iodination of low iodine thyroglobulin (preparation III) in vitro with carrier-free ¹³¹I plus varying amounts of ¹²¹I. The four diagrams, from top to bottom, represent iodination at 0, 20, 40, or 60 atoms of iodine/molecule of thyroglobulin (M₀ = 660,000), followed by reduction with mercaptoethanol and electrophoresis in 0.1% SDS. Each diagram is a composite. The portion to the right of the dashed vertical line is from gels of 8% polyacrylamide, and is expressed as the percentage of thyroglobulin’s ¹³¹I found in each gel segment of 2.5 mm. The portion to the left of the dashed vertical line represents an expanded scale of the first 15 mm of the 8% gel; for this, another aliquot of the sample was run on a 3.5% gel; the first 40 mm from the origin, shown to be equivalent to the first 15 mm of the 8% gels by molecular weight markers, was cut into equal segments of 5 mm, and each segment’s percentage of thyroglobulin’s ¹³¹I is shown in the diagrams.
iodine, there was a decrease in the iodothyronine content of thyroglobulin's thyroxine (56%) was in 26,000-dalton iodothyronine. The abscissa shows the atoms of iodine added per molecule of thyroglobulin. Most of iodine was in 26,000-dalton iodothyronine at each level of iodination (Fig. 4). After 40 atoms of added iodine, there was a decrease in the iodothyronine content of 26,000-dalton and an increase in that of 18,000-dalton iodothyronine. For thyroglobulin and 230,000-dalton iodopeptide, the formation of both iodotyrosines and iodothyronines was nearly linear with respect to iodine addition. The sum of 230,000-, 45,000-, 26,000-, and 18,000-dalton iodopeptides accounted for 98% of thyroglobulin's iodothyronine at 20 atoms of added iodine, 85% at 40 atoms, and 69% at 50 atoms. In the experiment shown in Table III and Fig. 4, the maximum iodothyronine content of 26,000-dalton iodopeptide was 0.97 residue/mol of 660,000; in an additional iodination experiment the maximum value was 0.81.

Eight other human thyroglobulin preparations, with basal iodine contents ranging from 0.02% to 0.62%, were each iodinated at several levels of 125I addition plus 125I tracer. Each showed the same major iodinated peaks that were described in Figs. 1 and 2. 125I-labeled 26,000-dalton iodopeptide reached a peak, in terms of its share of the 125I incorporated into thyroglobulin, at a total iodine content of 12 to 36 atoms/molecule of thyroglobulin. The 18,000-dalton iodopeptide was most prominent when the total iodine content of thyroglobulin was more than 40 atoms/molecule of thyroglobulin.

**Pulse Labeling—Goiter thyroglobulin (preparation II) was iodinated with a tracer of 125I, and then further iodinated with varying amounts of 125I (Fig. 5). Most of the initial iodination was on 230,000- and 45,000-dalton iodopeptides as before, with the former somewhat lower and the latter higher in this experiment. Further addition of iodine was accompanied by a decrease in the 125I content of 230,000- and 45,000-dalton iodopeptides. The 26,000-dalton iodopeptide rose from 5% of thyroglobulin's 125I when tracer alone was added, to a peak of 18% at 20 atoms of added iodine. By 40 atoms of added iodine, 26,000-dalton iodopeptide's 125I had decreased, and 125I appeared in 18,000-dalton iodopeptide. A peak of >300,000 iodopeptide (not shown in Fig. 5) first appeared at 40 atoms of added iodine, when it contained 8.9% of thyroglobulin's 125I, with no change at 60 atoms of added iodine (8.4%). A pattern similar to that of Fig. 5 was found in several other pulse-labeling experiments with this thyroglobulin preparation.**

Table IV shows the 125I-iodothyronine content of thyroglobulin and its major components at each level of iodine addition in the experiment shown in Fig. 5. In thyroglobulin, tracer 125I was converted from iodotyrosines to iodothyronines with successive additions of iodine, and a similar trend was seen with 230,000- and 45,000-dalton iodopeptides. There was little 125I-
Favored Sites for Thyroxine Formation

26,000-dalton iodopeptide on initial iodination, but it had a high content of iodothyronines. Once 18,000-dalton iodopeptide appeared in appreciable amounts, at 40 atoms of added iodine, it had most of its 125I as iodothyronines. For thyroglobulin, and 45,000- and 18,000-dalton iodopeptides, there was a marked increase in the ratio of T4 to T3 above 30 atoms of added iodine, and for thyroglobulin and 26,000-dalton iodopeptide this was not accompanied by an increase in its 125I-iodothyronine content.

We examined the possibility that some of the changes shown in Fig. 5 could result from simple exchange between the 125I and 127I isotopes. In one experiment, we iodinated thyroglobulin (preparation III) with 60 atoms of 125I/molecule, and after dialysis, performed a second iodination with carrier-free 125I. No 125I was found in 26,000-dalton iodopeptide, and less than 2.5% in 18,000-dalton iodopeptide. These values are consistent with new iodination at this level of added iodine; if there were complete iodine exchange, they would be approximately 12% and 16% respectively, from the data of Fig. 3. In a second experiment, we iodinated thyroglobulin from preparation I with carrier-free 125I, and found 2% of the isotope in 26,000-dalton iodopeptide, a figure compatible with new iodination of 26,000-dalton iodopeptide for this thyroglobulin preparation of 0.62% iodine (approximately 32 atoms of iodine/molecule). If there were no exchange with the 127I already in 26,000-dalton iodopeptide, this figure should have been 16.9% from the data of Table I.

Changes in gel pattern with different incubation periods during second iodination.

<table>
<thead>
<tr>
<th>Component</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>11</td>
<td>21</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>230,000-dalton</td>
<td>13</td>
<td>8</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>45,000-dalton</td>
<td>6</td>
<td>12</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>26,000-dalton</td>
<td>41</td>
<td>63</td>
<td>61</td>
<td>55</td>
</tr>
<tr>
<td>18,000-dalton</td>
<td>8</td>
<td>21</td>
<td>12</td>
<td>21</td>
</tr>
</tbody>
</table>

*Each entry is the percentage of the gel's total 125I found in the iodopeptides.

The human 26,000-dalton iodopeptide, like the rabbit 20,000- and 15,000-dalton iodopeptides show several similarities to the 20,000- and 15,000-dalton iodopeptides of small molecular size. From our data, these iodopeptides are unlikely to be the products of postmortem proteolysis. The 26,000- and 18,000-dalton iodopeptides show several similarities to the 20,000- and 15,000-dalton iodopeptides we have described in rabbit thyroglobulin (2). The human 26,000-dalton iodopeptide, like the rabbit 20,000-dalton iodopeptide, has an aspartic acid NH2 terminal, a high

DISCUSSION

The present work shows that human thyroglobulin contains thyroxine-rich iodopeptides of small molecular size. From our studies with surgically removed tissue and enzyme inhibitors, these iodopeptides are unlikely to be the products of postmortem proteolysis. The 26,000- and 18,000-dalton iodopeptides show several similarities to the 20,000- and 15,000-dalton iodopeptides we have described in rabbit thyroglobulin (2).
thyroxine content, and represents approximately 3-4% of thyroglobulin’s weight, or about 1 mol/mol of thyroglobulin. It contains one-fourth of thyroglobulin’s thyroxine. The 18,000-dalton iodopeptide, like the rabbit 15,000-dalton iodopeptide, has much less of thyroglobulin’s iodine than the 26,000-dalton iodopeptide, but most of that iodine is in the form of iodothyronine. We find iodothyronine-rich fractions of 20,000- to 26,000-dalton iodopeptides and of 12,000- to 18,000-dalton iodopeptides in thyroglobulins from fish, amphibia, reptiles, birds, and several mammals, in fact, in all species we have examined. Thus, these components are a consistent and probably important feature of the thyroglobulin molecule.

The studies in vitro with progressive addition of iodine to low iodine thyroglobulin show that 230,000- and 45,000-dalton iodopeptides are the first components to be iodinated, followed by 26,000-dalton iodopeptide and still later, 18,000-dalton iodopeptide. At moderate to low levels of iodine addition (10 to 20 atoms/molecule of thyroglobulin), most of the newly formed iodothyronine is in 25,000-dalton iodopeptide. With incremental additions of iodine, the iodothyronines in 26,000-dalton iodopeptide reached a peak of about 1 residue/molecule of thyroglobulin and then declined, while the diiodothyrosine and 3-iodothyrosine of 26,000-dalton iodopeptide increased steadily. Triiodothyronine represented a greater fraction of the iodothyronine content at lower iodination levels than at higher. These findings suggest that 26,000-dalton iodopeptide has a limited number, probably one, of iodothyronine forming sites; that it will form triiodothyronine or thyroxine, depending on whether the initial iodination of the outer ring produces 3-iodothyrosine or diiodothyrosine, which in turn depends on iodine availability; and that subsequent iodination of 26,000-dalton iodopeptide at higher iodine levels will be on tyrosyls other than the favored iodothyronine-forming one.

The pulse-labeling experiments suggest that iodine initially attached to 230,000-dalton iodopeptide later appears in 26,000-dalton iodopeptide, as more iodine is added to thyroglobulin, and that this is not simply an exchange between iodine isotopes. Among possible explanations for this finding are: (a) formation of diiodothyrosine on ~230,000-dalton iodopeptide with subsequent coupling of its diiodophenyl ring with a diiodotyrosyl on pre-existing 26,000-dalton iodopeptide; and (b) formation of thyroxine initially on ~230,000-dalton iodopeptide, with subsequent or simultaneous splitting off of a 26,000-dalton iodopeptide-sized peptide containing the thyroxine. At present, we favor the latter possibility because 26,000-dalton iodopeptide always has a high iodothyronine content, suggesting that the presence of iodothyronine is a condition for the occurrence of 26,000-dalton iodopeptide, or that they both result simultaneously from a common stimulus. Also, we have found, in another low iodine thyroglobulin (3 atoms of iodine/molecule of thyroglobulin), a progressive increase in 26,000-dalton iodopeptide’s share of thyroglobulin’s peptide material with increasing additions of iodine in vitro, reaching a peak at 20 atoms of added iodine/molecule. 

Iodinated 18,000-dalton iodopeptide was not found until thyroglobulin was well iodinated, above 0.6% iodine content by weight. This is still within the physiologic range of from 0.2 to 1.0% of thyroglobulin’s weight as iodine, or 10 to 50 atoms of iodine/molecule. As 18,000-dalton iodopeptide was iodinated, 26,000-dalton iodopeptide’s content of both iodine and thyroxine decreased, a pattern found consistently in both continuous and pulse-labeling iodination experiments. We do not have enough information to determine whether the changes in 18,000-dalton iodopeptide are directly related to those in 26,000-dalton iodopeptide.

The in vitro model for enzymatic iodination of thyroglobulin has been studied extensively by others (1, 8, 10, 11), and appears to reflect the major steps occurring in vivo. Lactoperoxidase produces results similar to those with thyroid peroxidase (8). Others (11), working with thyroid peroxidase, have noted the production of 3,3',5'-triiodothyronine, which we did not find in the current experiments. Most of our in vitro studies were with thyroglobulin from goiterers, the only sources available with low iodine content. In previous work on some of these thyroglobulin samples, changes in the amino acid composition suggested abnormalities in thyroglobulin structure (4). In the present experiments, all thyroglobulin samples from benign goiters appeared capable of forming iodothyronines on 26,000- and 18,000-dalton iodopeptides under our in vitro conditions. Any of the previously described abnormalities in structure, then, did not appear to interfere with hormone formation in vitro. The products of in vitro iodination (preparation I) were similar to those found in normal thyroglobulin (preparation I), and thus, this system seems a reasonable model for iodination in vivo.

Previously reported effects of iodination on the structure of thyroglobulin include an increase in density (12), an increased stability of the 19 S species (13), and a decrease in its free sulfhydryl content (14). These are consistent with the apparent increase in >300,000-dalton iodopeptide with iodination in the present experiments. We do not yet know how the changes in thyroglobulin stability with iodination are related to the production of the iodopeptides described in the present study. Post-translational (but antemortem) cleavage is an attractive possibility for their genesis (2), and does not appear to result from the thyroidal lysosomal enzymes studied thus far (15). Iodinating systems, such as exist in the thyroid and were used in vitro here, can cleave small tryptophan peptides (16) and can also split tryptophanyl bonds in lysozyme (17). Bacterial killing by myeloperoxidase and halide also involves peptide cleavage (18). As pointed out by Alexander (16), the thyroid contains the necessary components for a similar action against thyroglobulin, and iodination cleavage may be a step in its physiologic degradation. The present work is compatible with such a possibility and is being used as the basis for more extensive studies.

REFERENCES


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A. D. Dunn, unpublished work.
Favored Sites for Thyroxine Formation

Favored sites for thyroid hormone formation on the peptide chains of human thyroglobulin.
J T Dunn, P S Kim and A D Dunn


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