Noncovalent Interactions of a 26,000-dalton Peptide with 19 S Human Thyroglobulin*

Annalisa Tanini and Sidney Shifrin†

From the Section on Biochemistry of Cell Regulation, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205 and the Division of Cancer Biology and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

(Received for publication, April 22, 1983)

Extensive succinylation of 19 S normal human thyroglobulin having a high iodine content results in the formation of a 26,000-Da peptide. One-half mole of the peptide is obtained from 1 mol of the high molecular weight glycoprotein. The dissociation of the peptide is accompanied by the appearance of an intense absorption band which has a maximum at 264 nm. The absorption band is associated exclusively with the 26,000-Da peptide. The amino acid composition of the peptide differs from 19 S thyroglobulin by having no cysteine and higher contents of serine, alanine, tyrosine, phenylalanine, lysine, glycine, isoleucine, and histidine. The peptide also has a high thyroxine content. There were no detectable carbohydrates in the peptide.

The fluorescence spectrum of the 26,000-Da peptide shows an emission maximum at 405 nm which we have recently assigned to iodotyrosine-iodotyrosine interactions (Shifrin, S., Consiglio, E., and Kohn, L. D. (1983) J. Biol. Chem. 258, 3780–3786). A 26,000-Da peptide with the same physicochemical properties is found in extracts of normal human thyroid glands.
tandem cuvettes according to the method described previously (9).

Emission spectra were recorded on a Hitachi Perkin-Elmer spectrophluorometer, model MPF 4A, using microcuvettes. The cell compartment was maintained at a constant temperature of 20 °C.

Sialic acid content was determined by adding 100 units/ml of Vibrio cholerae neuraminidase to the glycoprotein contained in 0.1 M sodium acetate buffer, pH 5.5. A few drops of toluene were added prior to incubating at 37°C for 16 h. The amount of sialic acid removed by neuraminidase was determined by the fluorometric thio-barbituric acid assay (11).

The iodine content in the glycoproteins and glycopeptides was determined by the method of Palumbo et al. (12).

The protein concentration of 19 S thyroglobulin was determined at 280 nm using an extinction (ε280) of 10.0 (8).

The protein concentration of the other proteins were determined by the Lowry method (13).

Gel Electrophoresis—Analytical gels were run in 10% polyacrylamide containing 0.1% sodium dodecyl sulfate. A number of molecular weight markers were used. Gels were stained with Coomassie blue R-250 and Rf values were calculated relative to a bromphenol blue tracker dye band.

Thyroxine and triiodothyronine content in the proteins and in the peptide were measured by radioimmunoassay after treatment with pronase according to the method of Inoue and Taurog (14).

RESULTS AND DISCUSSION

Appearance of a New Absorption Band following Succinylation—Succinic anhydride can react with the ε-amino group of lysyl residues, with the hydroxyl groups of serine and threonine (15), and with the phenolic hydroxyl groups of tyrosine (16). In general, this modification does not markedly alter the ultraviolet absorption spectrum except for some small spectral shifts that arise from conformational changes in the polypeptide backbone (9). However, when succinic anhydride was added to 19 S human thyroglobulin, major changes occurred in the ultraviolet absorption spectrum. In order to best demonstrate these changes, we will first show the absorption spectrum of native 19 S human thyroglobulin (solid curve, Fig. 1A). The region of the spectrum from 300 to 330 nm reflects the iodotyrosine content of the large glycoprotein and this varies with the preparation of thyroglobulin.

Upon the addition of the modifying reagent at an input ratio of 1:1 for succinic anhydride:lysine, there was a marked change in the ultraviolet absorption spectrum (dashed curve, Fig. 1A). There is no change in the 300 to 330 nm region, but there is a marked increase in absorbance below 300 nm which

![Fig. 1. Changes in the ultraviolet absorption spectrum in the presence of succinic anhydride. A, native 19 S human thyroglobulin (19SthTg) (---); in the presence of a 1:1 input ratio of succinic anhydride:lysine (---); and in the presence of a 2:1 input ratio of succinic anhydride:lysine (-----). The concentration of 19 S human thyroglobulin was 0.5 mg/ml in 0.5 M sodium bicarbonate buffer, pH 8.1. B, ultraviolet difference spectrum: 1:1 succinyl thyroglobulin versus thyroglobulin (---); 2:1 succinyl thyroglobulin versus thyroglobulin (-----). The concentration of human thyroglobulin was 0.5 mg/ml in 0.5 M sodium bicarbonate.](http://www.jbc.org/)

![Fig. 2. Elution profile from Sepharose 4B column. 19 S human thyroglobulin (---); 20:1 input ratio of succinic anhydride:lysine (-----); 50:1 input ratio of succinic anhydride:lysine (-----). The column was 6 x 90 cm. The elution buffer was 0.01 M ammonium bicarbonate buffer, pH 7.4.](http://www.jbc.org/)
peptide has such a high content of succinate, we have all of the lysine, serine, and threonine residues are succinylated. However, 1000 residues of the 26,000-Da peptide contain 800 residues of succinate. It is clear that this radioactivity cannot be accounted for solely by the higher content of these amino acids in the peptide. In an effort to explain why the peptide has such a high content of [14C]succinate, we have tentatively assumed that the peptide contains functional groups which will react with succinic anhydride and may be part of the 264 nm chromophore. The structure of the chromophore is under investigation.

Absorption Spectra of the Separated Fractions—The ultraviolet absorption spectrum of thyroglobulin without peptide is shown by the solid curve in Fig. 4. Although the absorption spectrum of this glycoprotein has many features in common with the absorption spectrum of native 19 S human thyroglobulin, the differences between the two curves will be discussed shortly.

The absorption spectrum of the 26,000-Da peptide is shown by the dashed curve in Fig. 4. This spectrum is similar to the one that was predicted from the difference spectrum shown in Fig. 1B. These results demonstrate that the absorbance at 264 nm is associated exclusively with the 26,000-Da peptide. At the present time, it is not clear why the band at 264 nm is not expressed in native 19 S thyroglobulin but appears only when the 26,000-Da peptide begins to dissociate from the larger glycoprotein.

In the upper portion of Fig. 5, we compare the absorption spectrum of native 19 S human thyroglobulin (solid curve) with the spectrum of a solution of the high molecular weight fraction from 20:1 succinyl thyroglobulin, i.e. thyroglobulin without peptide (dashed curve) having the same absorbance at 280 nm. The difference between these two spectra is best shown by the difference spectrum shown in the lower portion of Fig. 5 using an expanded scale. This difference spectrum shows that thyroglobulin without peptide has a lower content of a component which is characterized by an absorption maximum at 310 nm. Gemmil (17) has summarized the absorption maxima of thyroxine and its derivatives together with their extinction coefficients and the pKₐ of their phenolic hydroxyl groups. Based on the data presented by Gemmil (17), we tentatively conclude that a peptide containing 11 diiodotyrosyl residues is lost from native 19 S human thyroglobulin as a result of succinylation.

Fluorescence Spectra—The solutions which were used to record the absorption spectra shown in Fig. 4 were also used to record fluorescence spectra (Fig. 6). The fluorescence spectrum of succinylated thyroglobulin without peptide is shown by the solid curve in Fig. 6. The emission maximum of this glycoprotein is at 338 nm which is a shift of 8-10 nm to longer wavelengths compared with the emission maximum of native 19 S human thyroglobulin (data not shown). Since the emission maximum of tryptophan in proteins is a sensitive measurement of the extent of modification, the fluorescence spectrum of the 26,000-Da peptide is also shown in Fig. 6.

The amino acid composition of the 26,000-Da peptide and of thyroglobulin without peptide are summarized in Table II. The results demonstrate that the peptide is markedly enriched in the thyroid hormones while the thyroglobulin without peptide is partially depleted of triiodothyronine and thyroxine.

The amino acid composition of the 26,000-Da peptide and of thyroglobulin without peptide are reported in Table II. The amino acid composition of these amino acids in thyroglobulin without peptide. These results are consistent with the composition of the peptides reported by Rolland and Lissitzky (4) for the low molecular weight fractions obtained after dissociation.

In an effort to determine the extent of succinylation of the peptide as well as of thyroglobulin without peptide, we carried out the modification reaction using an input ratio of [14C]succinic anhydride:lysine of 50:1. The reaction products were separated on the Sepharose 4B column and each fraction was examined for its [14C] content and absorbance at 280 nm.

There are 125 residues of [14C]succinate/1000 amio acid residues of thyroglobulin without peptide which indicates that all of the lysine, serine, and threonine residues are succinylated. However, 1000 residues of the 26,000-Da peptide contain 800 residues of [14C]succinate. It is clear that this radioactivity cannot be accounted for solely by the higher content of these amino acids in the peptide. In an effort to explain why the peptide has such a high content of [14C]succinate, we have tentatively assumed that the peptide contains functional groups which will react with succinic anhydride and may be part of the 264 nm chromophore. The structure of the chromophore is under investigation.

TABLE I
The triiodothyronine (T₃) and the thyroxine (T₄) content of 19 S thyroglobulin and its succinylated products

<table>
<thead>
<tr>
<th></th>
<th>T₃ (%)</th>
<th>T₄ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 S thyroglobulin</td>
<td>0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>Thyroglobulin without peptide</td>
<td>0.0049</td>
<td>0.10</td>
</tr>
<tr>
<td>26,000-Da peptide</td>
<td>0.16</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Absorption Spectra of the Separated Fractions—The ultraviolet absorption spectrum of thyroglobulin without peptide is shown by the solid curve in Fig. 4. Although the absorption spectrum of this glycoprotein has many features in common with the absorption spectrum of native 19 S human thyroglobulin, the differences between the two curves will be discussed shortly.

The absorption spectrum of the 26,000-Da peptide is shown by the dashed curve in Fig. 4. This spectrum is similar to the one that was predicted from the difference spectrum shown in Fig. 1B. These results demonstrate that the absorbance at 264 nm is associated exclusively with the 26,000-Da peptide. At the present time, it is not clear why the band at 264 nm is not expressed in native 19 S thyroglobulin but appears only when the 26,000-Da peptide begins to dissociate from the larger glycoprotein.

In the upper portion of Fig. 5, we compare the absorption spectrum of native 19 S human thyroglobulin (solid curve) with the spectrum of a solution of the high molecular weight fraction from 20:1 succinyl thyroglobulin, i.e. thyroglobulin without peptide (dashed curve) having the same absorbance at 280 nm. The difference between these two spectra is best shown by the difference spectrum shown in the lower portion of Fig. 5 using an expanded scale. This difference spectrum shows that thyroglobulin without peptide has a lower content of a component which is characterized by an absorption maximum at 310 nm. Gemmil (17) has summarized the absorption maxima of thyroxine and its derivatives together with their extinction coefficients and the pKₐ of their phenolic hydroxyl groups. Based on the data presented by Gemmil (17), we tentatively conclude that a peptide containing 11 diiodotyrosyl residues is lost from native 19 S human thyroglobulin as a result of succinylation.

Fluorescence Spectra—The solutions which were used to record the absorption spectra shown in Fig. 4 were also used to record fluorescence spectra (Fig. 6). The fluorescence spectrum of succinylated thyroglobulin without peptide is shown by the solid curve in Fig. 6. The emission maximum of this glycoprotein is at 338 nm which is a shift of 8-10 nm to longer wavelengths compared with the emission maximum of native 19 S human thyroglobulin (data not shown). Since the emission maximum of tryptophan in proteins is a sensitive measurement of the extent of modification, the fluorescence spectrum of the 26,000-Da peptide is also shown in Fig. 6.

The amino acid composition of the 26,000-Da peptide and of thyroglobulin without peptide are summarized in Table II. The results demonstrate that the peptide is markedly enriched in the thyroid hormones while the thyroglobulin without peptide is partially depleted of triiodothyronine and thyroxine.

The amino acid composition of the 26,000-Da peptide and of thyroglobulin without peptide are summarized in Table II. The results demonstrate that the peptide is markedly enriched in the thyroid hormones while the thyroglobulin without peptide is partially depleted of triiodothyronine and thyroxine.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>26,000-Da peptide</th>
<th>Thyroglobulin without peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>87</td>
<td>26</td>
</tr>
<tr>
<td>Threonine</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Serine</td>
<td>132</td>
<td>82</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>116</td>
<td>115</td>
</tr>
<tr>
<td>Proline</td>
<td>13</td>
<td>56</td>
</tr>
<tr>
<td>Glycine</td>
<td>180</td>
<td>117</td>
</tr>
<tr>
<td>Alanine</td>
<td>145</td>
<td>90</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Valine</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Methionine</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Leucine</td>
<td>89</td>
<td>94</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Lysine</td>
<td>55</td>
<td>4</td>
</tr>
<tr>
<td>Histidine</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Arginine</td>
<td>40</td>
<td>45</td>
</tr>
</tbody>
</table>

TABLE II
The amino acid composition of the 26,000-Da peptide and thyroglobulin without peptide

Data are expressed as residues/1000 residues.
iodotyrosine content of the peptide. The tryptophan emission maximum is at 332 nm which is very close to the emission maximum of native 19S human thyroglobulin. This is surprising in view of our observation that the peptide is extensively succinylated, which should result in unfolding of the polypeptide chain.

A second emission band with a maximum at 405 nm is seen in the fluorescence spectrum of the 26,000-Da peptide. This spectrum is similar to the one, which we recently reported for highly iodinated 27S amannosyl thyroglobulin (7). The conditions under which the 405 nm band appeared in that study are also present in the peptide. That is, there is a high local concentration of iodotyrosyl residues and there are no carbohydrate chains. Based on model studies using a co-polymer of lysine:iodotyrosine, we suggested that the emission maximum at 405 nm resulted from iodotyrosyl-iodotyrosyl interactions (7).

Evidence is mounting that the strength of iodotyrosyl-iodotyrosyl interactions is sufficiently strong to account for the aggregating properties of iodinated thyroglobulin (19-25). Thus, thyroglobulin molecules with a low iodine content have a high content of 12S subunits while a high iodine content favors the formation of 27S iodoprotein (19, 25). Similarly, iodotyrosine-iodotyrosine interactions may be responsible for maintaining the native conformation or the highly succinylated 26,000-Da peptide.

Existence of the 26,000-Da Peptide in Thyroid Extracts—We have found that extracts of normal thyroid glands contain the 26,000-Da peptide as a very small percentage of the total amount of protein which can be extracted. The elution pattern of the 26,000-Da peptide obtained after succinylation of human thyroglobulin is shown by the solid curve in Fig. 7, while an extract of normal thyroid glands gives the elution pattern shown by the dashed curve. The 26,000-Da peptide from the extracts exhibited the same absorption spectrum as was found upon succinylation with a maximum at 264 nm. An extract of thyroid goiters also contains a peptide with M = 40,000, but its absorption spectrum has the usual maximum at 280 nm, not at 264 nm. The 264 nm band is associated exclusively with the 26,000-Da peptide. The fluorescence spectrum of the 26,090-Da peptide that is extracted directly from the human thyroid gland also has maxima at 330 and 405 nm, identical with the dashed curve in Fig. 6.

The 26,000-Da peptide that is isolated directly from normal human thyroid glands has no detectable sugar residues and has the same iodine content as the peptide released by extensive succinylation. In fact, the 26,000-Da peptide obtained either by direct extraction of the thyroid glands or by succinylation of 19S human thyroglobulin has the same chemical and physicochemical properties.

Although the physicochemical properties of thyroglobulin have been extensively studied, its subunit composition re-

FIG. 4. Ultraviolet absorption spectrum. Thyroglobulin without peptide (——); 26,000-Da peptide from 20,1 succinyl thyroglobulin (——).
Da peptide obtained from succinylation of human thyroglobulin (\(-\ -\)). This is not detectable in intact 19 S thyroglobulin, nor is the peptide released from normal 19 S human thyroglobulin. The peptide may be the active site of thyroid hormone formation. The peptide can be released from normal 19 S human thyroglobulin in the 26,000-Da peptide, which is enriched in triiodothyronine. This peptide is found in other peptides in the thyroid extracts. This peptide also has a fluorescence maximum at 405 nm due to iodotyrosyl-iodotyrosyl interactions.

The unique feature of the 26,000-Da peptide is its ultraviolet absorption spectrum with a maximum at 264 nm. We are currently attempting to determine the molecular structure of the 264-nm chromophore and to examine a possible role for this group in the biosynthesis of thyroid hormones on the 26,000-Da peptide. We previously suggested (7) that iodotyrosine-iodotyrosine interactions such as those which exist in the 26,000-Da peptide, may facilitate the formation of thyroxine and triiodothyronine. Thus, the 26,000-Da peptide may be the active site of thyroid hormone formation. The peptide may also be stabilized and stored in the thyroid follicle in association with the high molecular weight 19 S glycoprotein.

In summary, we have demonstrated that the 26,000-Da peptide can be released from normal 19 S human thyroglobulin which is identical with the peptide isolated directly from human thyroglobulin. This peptide is enriched in triiodothyronine and thyroxine and has an absorption maximum at 264 nm which is not detectable in intact 19 S thyroglobulin, nor is it found in other peptides in the thyroid extracts. This peptide also has a fluorescence maximum at 405 nm due to iodotyrosyl-iodotyrosyl interactions.
Noncovalent interactions of a 26,000-dalton peptide with 19 S human thyroglobulin.
A Tanini and S Shifrin


Access the most updated version of this article at http://www.jbc.org/content/258/20/12553

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/20/12553.full.html#ref-list-1