IODOTHYRONINE CONTENT
OF HUMAN THYROID ALBUMIN

By
Colette Thomas-Morvan

ABSTRACT

Stable thyroid hormones (T₄ and T₃) have been demonstrated in pure albumin isolated from normal human thyroid tissue iodinated in vivo. Five samples of albumin were separated from other thyroid proteins by acrylamide gel electrophoresis. After pronase hydrolysis, the content of thyroid hormones was measured chemically (T₄ + T₃) as well as by competitive radioactive measurement (T₄) and radioimmunoassay (T₃). The purity of the albumin and validity of these measurements were confirmed by different techniques.

The synthesis of thyroid hormones is not therefore a property unique to Tg and may occur in albumin. However the amount of iodothyronines in the albumin (average 0.004 residue per molecule) is much less than that found in Tg (0.5 residue per molecule). In the albumin as in Tg the number of hormone residues per molecule is proportional to the number of atoms of iodine. At an equivalent iodine concentration, the albumin seems capable of forming the thyroid hormones as well as Tg. The difference between these two proteins, in their capacity to synthesize thyroid hormones, seems to depend on their capacity for iodination. This difference of iodination does not seem to be linked with the number of tyrosyl residues, but might be related to the position of these residues.

Soluble thyroid proteins contain, apart from thyroglobulin (Tg) and its sub-units, an albumin fraction the possible role of which in hormone synthesis is a matter of controversy. It has been demonstrated previously that this albumin is not

Abbreviations

1) Tg: thyroglobulin; MIT: 3-monoiodotyrosine; DIT: 3,5-diiodotyrosine; T₃: 3,5,3'-triiodothyronine; T₄: thyroxine.
synthesised by the human thyroid tissue maintained in organotypic culture and that it incorporates radioiodine (Ceriani et al. 1971). In certain congenital goitres in which Tg is absent, the thyroid hormones are nevertheless synthesised, which suggests that albumin can act as the site of hormone synthesis (Lissitzky et al. 1967, 1968; Tubiana et al. 1968; Savoie et al. 1973; Thomas-Morvan et al. 1973). In the present study, we extracted the albumin from normal human thyroid tissue by acrylamide gel electrophoresis, and measured its content of thyroid hormones.

MATERIALS AND METHODS

Isolation of the albumin

Normal human thyroid tissue was taken from the relatively normal areas of thyroid gland excised for adenomas. The thyroid tissue specimen (about 1 g) was homogenised in Tris-HCl buffer (0.05  m Tris-0.1  m NaCl) as soon as possible after removal by surgery. The homogenate was centrifuged at 4000  g for 20 min at 4°C. The supernatant was electrophorised on 7 % acrylamide gel (Davis 1964) using a slight modification of the method described previously (Ceriani et al. 1971): the tubes were not completely filled with gel, so that a space of about 1 ml was left empty, this was then filled with buffer and the tube closed with a dialysis membrane. The electrophoresis was made until the albumin, stained with bromophenol blue in the electrophoresis buffer, migrated beyond the gel. By this method the albumin solution, retained by the dialysis membrane, was recovered, then isolated from lighter proteins (especially haemoglobin stained red) which migrated more slowly than albumin and remained in the gel. Five samples of albumin from 5 different normal thyroids were isolated by this method.

Measurement of stable T4 and T3

The protein content of these solutions was measured by UV absorption at 280 nm. 127I was measured with a Technicon autoanalyser (Comoy 1967). The albumin solutions were hydrolysed at pH 8 with pronase for 6 h at 37°C. On these hydrolysates, three different techniques were used: T4 + T3 were extracted with Bio-Rad resin columns and their iodine content was measured (Pileggi & Kessler 1968); determination of T4 was also carried out by radioisotopic competition (Murphy & Pattee 1964) using a Tetrasorb-Kit (Abbott), and T3 was determined by radioimmunoassay (Nouel et al. 1973). The same measurements were made for comparison on two samples of stable Tg recovered from human thyroid extracts after ultracentrifugation on a sucrose gradient (5–20 %).

Study of possible contamination of albumin by small fragments of Tg during the isolation of the albumin

131I labelled Tg, prepared by sucrose gradient ultracentrifugation from normal human thyroid labelled with 131I in vivo and in vitro, was mixed with stable albumin. When in vivo 131I-labelled Tg was used, 250  mCi of 131I was administered 24 h before surgery. The albumin was later isolated and harvested by acrylamide gel electrophoresis and its radioactivity measured.
Study of possible contamination of albumin by T₄ residues synthetized in Tg and weakly bound to free sites on the surface of albumin

In order to differentiate the T₄ bound on the external sites of albumin from T₄ included within the albumin molecule, we determined by competition analysis the T₄ content in non-hydrolysed samples of albumin, and in the same samples after hydrolysis with pronase. Two samples were prepared from one human thyroid: the albumin was extracted by acrylamide gel electrophoresis, and a light protein fraction (3–8 S) was prepared by sucrose gradient ultracentrifugation.

Percentage of $^{125}$I ($T_4 + T_3$) in the light fraction (3–8 S) and Tg extracted from thyroid tissue labelled with $^{125}$I in culture

Specimens of normal human thyroids taken during surgery were maintained in organ culture for 2 days according to the method of Nataf et al. (1966). $^{125}$I was added to the medium 24 h before the end of the culture period. The fragments were then homogenized. After dialysis against Tris-HCl buffer, the extracts were ultracentrifuged on a sucrose gradient (5–20%). The Tg (19 S) and the light fraction (3–8 S) labelled with $^{125}$I were hydrolysed with pronase, then chromatographed on paper using collidine – 3% NH₄OH as the solvent.

RESULTS

The light fraction (3–8 S) labelled with $^{125}$I in culture, isolated by sucrose gradient ultracentrifugation from the soluble proteins of human thyroid tissues, contained mostly $[^{125}$I]MIT (about 77% of radioactive iodine incorporated in

<table>
<thead>
<tr>
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<th>$^{125}$I % on chromatograms</th>
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<tbody>
<tr>
<td></td>
<td>MIT</td>
</tr>
<tr>
<td>Light fraction (3–8 S)</td>
<td>79.4</td>
</tr>
<tr>
<td></td>
<td>76.4</td>
</tr>
<tr>
<td></td>
<td>74.4</td>
</tr>
<tr>
<td>Tg (19 S)</td>
<td>58.9</td>
</tr>
</tbody>
</table>

Three samples of light fraction (3–8 S) were isolated by sucrose gradient ultracentrifugation from 3 human normal thyroid tissues labelled with $^{125}$I in organ culture (2 days in culture, 24 h incorporation $^{125}$I). One sample of human normal Tg (19 S) was obtained using the same methods.
Acrylamide gel electrophoresis (7 %) of albumin obtained from human normal thyroid tissue. This albumin was separated from the other thyroid proteins using acrylamide gel electrophoresis (7 %), concentrated, then re-run in acrylamide gel electrophoresis. The gel was stained by amido-black.

Albumin after 24 h of culture in radioactive medium) (Table 1). However, small amounts of T₄ and T₃ labelled with ¹²⁵I were present (1 to 3 %). This percentage is only slightly less than that observed in Tg (Table 1).

The 3–8 S fraction was heterogeneous and contained several sub-fractions (Ceriani et al. 1971), then the albumin was isolated from the other thyroid proteins by electrophoresis. Five samples of pure albumin were thus extracted from 5 normal human thyroids. These solutions, when re-run on acrylamide gel electrophoresis, gave only a single band corresponding to serum albumin (Fig. 1). The purity of these albumin solutions was checked immunologically. By immunodiffusion in agar (Ouchterlony) they gave a precipitation band with an anti-human albumin anti-serum but no precipitation band with an anti-human Tg anti-serum.

Both chemical analysis and competition method demonstrated the presence of stable T₄ and T₃ in the albumin in about similar amounts (Table 2).
Table 2.
Stable iodothyronine content (residue per molecule of albumin) in 5 samples of human thyroid albumin.

<table>
<thead>
<tr>
<th>Albumin no.</th>
<th>T4 + T3 Chemical analysis</th>
<th>T4 competitive analysis + T3 radioimmunoanalysis</th>
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<tbody>
<tr>
<td>1</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>0.006</td>
<td>0.013</td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>5</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean</td>
<td>0.0034</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

Five samples of albumin were isolated by acrylamide gel electrophoresis, then pronase-digested. Stable iodothyronines were measured by chemical analysis (T4 + T3) and radioactive techniques: competitive analysis (T4) and radioimmunoassay (T3).

Table 3.
127I and stable iodothyronine content in albumin and Tg.

<table>
<thead>
<tr>
<th></th>
<th>127I μg per 100 μg protein</th>
<th>127I μg per molecule</th>
<th>(T4 + T3) μg per 100 μg protein</th>
<th>(T4 + T3) residue per molecule</th>
<th>127I % as (T4 + T3) residue per 100 residues tyrosine</th>
<th>(T4 + T3) residue per 100 residues tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (mean of 5 samples)</td>
<td>0.008</td>
<td>0.05</td>
<td>0.0039</td>
<td>0.003</td>
<td>33</td>
<td>0.25</td>
</tr>
<tr>
<td>± se</td>
<td>0.00008</td>
<td>0.007</td>
<td>0.0001</td>
<td>0.0003</td>
<td>5</td>
<td>0.04</td>
</tr>
<tr>
<td>Tg 1</td>
<td>0.05</td>
<td>2.8</td>
<td>0.028</td>
<td>0.24</td>
<td>34</td>
<td>2.5</td>
</tr>
<tr>
<td>Tg 2</td>
<td>0.18</td>
<td>9.5</td>
<td>0.055</td>
<td>0.47</td>
<td>20</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Albumin was isolated from thyroid gland by acrylamide gel electrophoresis, Tg by sucrose gradient ultracentrifugation. After hydrolysis by pronase, iodothyronines content was measured using chemical analysis. The 5 samples of albumin were obtained from human normal thyroid tissues, Tg 1 from thyroid adenoma, Tg 2 from normal thyroid.
The amounts of T₃ and T₄ were on an average 0.004 residue of (T₄ + T₃) per molecule of albumin, and 0.0008 residue of T₃ per molecule. The iodine content of albumin was very small (average 0.008 %, or 0.05 atom of ¹²⁷I per molecule of albumin) (Table 3). Table 3 also shows the content of hormones in Tg with a low content of iodine (0.05 %) extracted from a thyroid adenoma, and in Tg from normal human thyroid tissue (iodine content 0.18 %). The amount of hormones in the albumin fraction (in residues per molecule) is about 100 times smaller than that in Tg (Table 3). On the other hand, the average number of residues of T₄ or T₃ is approximately proportional to the mean number of iodine atoms per molecule of protein in both albumin and Tg (Figs. 2 and 3).

The possibility of contamination of the albumin by fragments of Tg not detectable immunologically was studied. A very slight contamination of albumin was observed after it had been mixed with [¹³¹I]Tg and then separated by acrylamide gel electrophoresis. Only 0.03 % of the total radioactivity of the Tg was recovered in the albumin fraction, this corresponding to 0.0002 atom of iodine per molecule of albumin. This contamination seemed to come from the liberation of small fragments of Tg during the electrophoresis, since this was observed when radioactive Tg was electrophorised alone on acrylamide gel.

**Fig. 2.**

Relationship between the number of iodine atoms per molecule of protein and the number of (T₄ + T₃) residues per molecule of protein. Iodothyronines were measured by chemical analysis after pronase hydrolysis. • albumin obtained from human normal thyroid tissues; ○ Tg extracted from thyroid adenoma; × Tg extracted from normal tissue.

293
These traces of contamination do not appear to result from the contact of Tg with albumin, since the percentage of radioactive contaminant was not correlated with the duration of the contact between the Tg and albumin before electrophoresis.

Possible contamination by T₄ molecules weakly bound to free external sites on the albumin was also tested. This contamination was very slight. The T₄ bound to albumin and recovered by competition analysis was 0.00014 µg per 100 µg of protein. This content was ten times higher after pronase hydrolysis (0.0018 µg of T₄ per 100 µg of protein). This result was also obtained with light fraction (3–8 S) prepared by ultracentrifugation (0.00020 µg of T₄ before hydrolysis, and 0.0020 µg after pronase hydrolysis). The validity of the competition method in the presence of similar amount of albumin was also checked. Five µg of T₄ was added to a solution of human serum albumin. The T₄ was measured by competition analysis before and after hydrolysis: The results were similar (5.0 µg in the non-hydrolysed solution, and 4.7 µg after hydrolysis).

**DISCUSSION**

The presence of albumin among the thyroid proteins in man under normal conditions has been reported by several investigators (Robbins et al. 1959;
Jonckheer 1963; Stanley 1964; Ramagopal et al. 1965; Shulman et al. 1967). This albumin is capable of being iodinated and of forming moniodotyrosine (MIT). Meanwhile the possibility of the synthesis of triiodothyronine (T₃) and thyroxine (T₄) in albumin is still a matter of controversy. This is mainly explained by the difficulty of isolation of albumin from thyroid tissue. Our technique of the separation of albumin from the other proteins present in the thyroid extracts (Tg, sub-units of Tg, haemoglobin) has enabled pure albumin to be isolated from normal thyroid tissue. In five samples of this albumin we measured the amount of T₃ and T₄ by three different methods and compared their values to those in Tg, expressing the results in residues per molecule of protein.

The validity of the techniques used for the isolation and the measurement was confirmed by the following results: the amount of hormones (T₄ + T₃) in normal Tg (0.47 residue of T₄ + T₃ for 9.5 atoms of ¹²⁷I per molecule) is similar to that found by previous investigators for human Tg having an equivalent iodine content (Rolland et al. 1972, 1973; Sorimachi & Ui 1974). In the five samples of albumin, as with the Tg, a fair agreement was obtained between the results of the hormone assays by chemical analysis and by radioactive methods using both competitive technique and radioimmunoassay. The albumin fraction recovered after acrylamide gel electrophoresis only gave a single band when subjected to a further electrophoresis, and was precipitated in agar immunodiffusion by a rabbit anti-human albumin anti-serum, but not by an anti-human Tg anti-serum. The light protein fractions other than albumin do not interfere with the measurement of the hormones since they are not iodinated (Ceriani et al. 1971). Traces of small fragments of Tg can be minor contaminants of albumin during electrophoresis but their level is very low (3 x 10⁻⁴) and do not interfere with the result of measurement of the T₄ + T₃ associated with albumin. Comparison of the amount of T₄ in the albumin before and after hydrolysis showed that only a small proportion (about 1/10th) of the T₄ is weakly bound to free sites on the surface of albumin. These findings agree with those of Dunn & Ray (1973) made on a human thyroid tumour. The major part of the T₄ is therefore incorporated in the albumin molecules.

Hence albumin appears capable of synthesising thyroid hormones and this property does not seem to be specific to Tg. It is important to note that in our experiments we used native albumin extracted from human thyroid. Various investigators have studied whether T₄ could be formed in albumin by using serum albumin iodinated in vitro. Conflicting results were obtained by these experiments (Perlman & Edelhoch 1967; Rolland et al. 1972, 1973). In the case of Tg, Ogawara et al. (1972) and Rolland et al. (1973) have both shown that the T₄ content is smaller after iodination in vitro than in vivo, for an equal iodine content. It is probable that it is the same for albumin. As in Tg, the synthesis of T₄ and T₃ in albumin might be effected more readily in vivo than
in vitro, so that this would explain why we have been able to find evidence for the synthesis of thyroid hormones in albumin iodinated in vivo.

The amount of residues of iodothyronines per molecule of albumin is much smaller than that in Tg (about 100 times less); this is in agreement with the idea that the efficiency of Tg for the formation of thyroid hormones is exceptionally high (Rolland et al. 1973; Tubiana et al. 1973). However our results show that, in albumin as in Tg, the average number of $T_4$ and $T_3$ residues per molecule of protein is proportional to the mean number of iodine atoms per molecule of protein, which is in agreement with the results obtained previously in Tg from normal and diseased thyroid tissues (Tubiana et al. 1973; Thomas-Morvan et al. 1973, 1974). Hence, the important factor seems to be the mean level of iodine and hormones per molecule. At an equivalent iodine concentration, albumin seems able to synthesize thyroid hormones as well as Tg. Moreover the percentage of $^{127}$I as hormones ($T_4 + T_3$) in albumin and in Tg is virtually equivalent (in the order of 30% (Table 3). This is in agreement with the hypothesis that we (Tubiana et al. 1968, 1973; Thomas-Morvan et al. 1973) and other investigators (Lissitzky et al. 1967, 1968; Savoie et al. 1973) have previously put forward for goitres without Tg. The fact that albumin on an average contained only 0.05 atom of iodine per molecule, when it needs a minimum of 4 atoms of iodine to obtain one residue of $T_4$, suggests that synthesis of one residue of $T_4$ results from the interaction of either several molecules of albumin, or albumin and either DIT or small free iodinated polypeptides. The coupling process does not actually seem to happen within the same molecule. The iodine content of pathological Tg suggests that a similar mechanism also must occur when Tg is involved.

The low content of hormones in thyroid albumin in relation to that in Tg (100 times weaker) may originate not from a difference of their synthetic activities but simply from the fact that the albumin is much less iodinated than Tg. In albumin, only one molecule among 20 contains one atom of $^{127}$I, whilst each molecule of “normal” Tg contains a mean of 10 atoms of $^{127}$I. The difference between these two proteins may reside in their capacity of iodination. Albumin contains less tyrosine than Tg (Spiro 1970); moreover at an equivalent tyrosine content, albumin is about thirty times less iodinated than Tg (Table 3). This difference in iodination does not seem to be linked with the number of tyrosyl residues, but it might be due to the position of these residues.

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