Iodinated Serum Proteins in Functional Thyroid Carcinoma

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Summary

Iodoproteins in the serum of a patient with functioning thyroid carcinoma and in the plasma and tumors of rats bearing transplantable functioning thyroid tumors were investigated by electrophoresis, density gradient ultracentrifugation, immunoprecipitation, and immunoelectrophoresis. Iodinated serum proteins were found in the blood and tumors in all cases studied and, in some, accounted for essentially all of the iodoprotein in the blood. Most of this material was iodinated serum albumin, but iodinated globulins were also detected. In the case of some rat tumors, particularly in Line 1-8, a large proportion of the iodoprotein in both the tumor and the blood was not precipitated by antiserum against serum protein and also differed from thyroglobulin. The nature of this abnormal iodoprotein was not established.

Introduction

In a large proportion of patients with functional thyroid carcinoma, the serum contains an iodoprotein (S-iodoprotein) that has many of the properties of serum albumin (6, 12, 20). In some patients this may account for almost all of the iodine in the blood. A similar iodoprotein has been found in human thyroid carcinoma extracts (1, 8, 14), where it constituted 16% or less of the soluble iodoprotein of the tumor. The serum S-iodoprotein in 3 thyroid carcinoma patients was differentiated from iodinated serum albumin by the finding that it did not react with antialbumin antiserum and was not protected from heat precipitation by caprylate (20).

The S-iodoprotein is not found only in thyroid carcinoma, since it has now been detected in blood in a variety of non-neoplastic thyroid diseases as well as in normal and diseased thyroid glands (9, 11). Furthermore, there have been several reports that some S-iodoproteins in man do react with antialbumin antibodies (2, 4, 19). The presence of iodinated endogenous serum albumin (3, 4), as well as the iodination of injected heterologous serum albumin (3, 15), has been demonstrated in animals. In some instances, iodinated γ-globulin has also been detected (4).

Because of these recent observations, the question of the identity of S-iodoprotein in thyroid carcinoma has been re-investigated. The findings in 1 patient with functional thyroid carcinoma and in 3 types of transplantable rat thyroid tumors (21) form the basis of this report.

Materials and Methods

The patient (D.P.) was a 22-year-old woman, who was known to have thyroid carcinoma since the age of 15 years. The thyroid gland had been totally removed at that time, with the histologic diagnosis of follicular adenocarcinoma. Three therapeutic doses of 131I (134—149 mc) had been administered during the next 2 years in an attempt to destroy miliary-type pulmonary metastases, which were very active in concentrating iodine. After a 5-year period, during which the metastases had been static and non-functional, they again began to grow and to accumulate iodine. An oral dose of 156 mc of 131I was administered for therapy, and serum was then obtained at intervals from 1 to 8 days later.

Five lines of transplantable rat thyroid tumors (21), which were generously provided by S. Wollman, are described in Table 1. Two of them (Lines 1-1c and 1-1c, slow) were from the same line that had been used earlier for studies on thyroid iodoproteins (13). They differed from each other histologically and in their rate of growth.

Each rat was given an injection i.p. of 300 μc of iodide-131I. After 48 hr, the animals were anesthetized with ether and killed by cardiac puncture and exsanguination into a heparinized syringe. The thyroid gland (attached to a segment of trachea) and the tumors were then removed. The tumors were frozen, sliced thinly, and extracted with 0.15 M NaCl (2 ml/gm) for about 15 hr at 4°C. The clear extract was obtained by centrifugation.

Ion-exchange chromatography of iodine compounds in serum or plasma was done on columns of Dowex 1-X2 by methods described elsewhere (5). Paper chromatography of iodine compounds in serum or plasma, or in the tumor extracts, was performed on Whatman No. 3MM paper by methods described elsewhere (5). Paper chromatography of iodine compounds in serum or plasma, or in the tumor extracts, was performed on Whatman No. 3MM paper, with the use of n-butanol-ethanol-0.5 N NH₄OH (5:1:2) or n-butanol-1,4-dioxane-2 N NH₄OH (4:1:5, upper phase). Zone electrophoresis was performed on Whatman No. 3MM paper with the use of tris(hydroxymethyl)aminomethane-maleate buffer (pH 8.6, ionic strength ~0.1) or on agar-coated microscope slides with the use of barbital buffer (pH 8.2, ionic strength 0.05). Conventional and reverse flow methods were used (10).

The sedimentation of serum or tumor extracts in a linear sucrose gradient was carried out in a Spinco model L ultracentrifuge with the SW 25 swinging-bucket rotor. The detailed procedure was recently described (15).

Immunoelectrophoresis was performed on agar-coated microscope slides with the use of conventional technics (17). Autoradiographs were prepared by opposing the dried but unstained slide to Kodak No-Screen X-ray film.

Immunoprecipitation was performed by adding the required amounts of antigen and antiserum in a total volume of 0.1—0.3 ml. Protein concentration in the antigen solution was estimated by measuring the absorbance at 280 μ, with the absorbance of whole serum used as a reference. The quantity of antigen was expressed as μl of antigen solution equivalent in protein con-
TABLE 1
TRANSPLANTABLE RAT THYROID TUMORS

<table>
<thead>
<tr>
<th>TUMOR LINE</th>
<th>GENERATION</th>
<th>TUMOR HISTOLOGY</th>
<th>TUMOR WT. (gm)</th>
<th>131I DISTRIBUTION AT 48 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1c</td>
<td>39</td>
<td>Cellular with occasional colloid droplets</td>
<td>2</td>
<td>Tumor (% dose)</td>
</tr>
<tr>
<td>1-1c, slow</td>
<td>37</td>
<td>Varying sized follicles containing colloid</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>1-8</td>
<td>40</td>
<td>Very small follicles containing colloid</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>16-6</td>
<td>34</td>
<td>Long narrow follicles with colloid</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>1-2</td>
<td>34</td>
<td>Cellular, vascular, no obvious follicles</td>
<td>14</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The antisera were purchased from Mann Research Laboratories, New York, or from Serpasteur, Pasteur Institute, Paris. Radioactivity measurements were carried out with a γ-scintillation counter.

RESULTS

Human Thyroid Carcinoma

Serum 131I was examined by paper and column chromatography to determine whether labeled iodoprotein was present. As indicated in Table 2, iodoprotein was detected on the 1st day. Because of the large proportion of iodide, quantitation of the iodoprotein (5% of total 131I or 50% of organic 131I) is rather uncertain. On subsequent days, the iodoprotein increased to 50% of the total 131I and was 58% of the organic 131I on the 4th day. Triiodothyronine was not detected. Although 2 zones of 131I were seen in the iodotyrosine area on autoradiograms, the identity of these with carrier monoiodotyrosine and diiodotyrosine was not certain.

Column chromatography was used to prepare iodoprotein and was not carried beyond the water-elution step. Paper chromatography of the iodoprotein fractions eluted from the Dowex columns revealed 131I only at the origin. Paper electrophoretic analysis of the column iodoprotein fractions revealed that 75% of the 131I was in the albumin zone; the remainder was found in 2 poorly separated zones in the β- and α-globulin regions.

TABLE 2
CHROMATOGRAPHY OF 131I IN THE SERUM OF PATIENT D.P.

| DAYS AFTER 131I | METHOD | % OF TOTAL 131I | Iodoprotein | Iodide | Thyrosine | Monoiodo- | Diiodo- |
|----------------|--------|-----------------|-------------|--------|----------| tyrosine  | tyrosine |
| 1              | P      | 6               | 88          | 3      | 3        |           |         |
| 3              | C      | 5               | 41          | 27     | 29       | 3         |         |
| 4              | P      | 5               | 50          | 14     | 29       | 7         |         |
| 8              | C      | 49              |             |        |          |           |         |

a P, paper chromatography (solvent, n BuOH - dioxane - NH4OH); C, column chromatography (Dowex 1-X2).

Origin in paper chromatography; H2O eluate in column chromatography.

See text.

Centrifugation to whole serum. The mixtures were incubated at room temperature for 2-4 hr and at 4°C for 15-24 hr. After centrifugation, the supernatant was removed by suction. Because of the small amount of antigen employed, it was not possible to wash the precipitate. In the absence of a specific precipitate, the unwashed tube contained about 5% of the total radioiodine.

CHART 1. Density gradient ultracentrifugation of dialyzed serum obtained from patient D.P. 4 days after 131I therapy. The zone labeled 19 S indicates the location of bovine thyroglobulin in a control tube during the same centrifuge run.
Density gradient ultracentrifugation was performed on whole serum of Days 2, 4, and 6, which had been dialyzed against 0.15 M NaCl to remove iodide. Ten mg of bovine thyroglobulin was added to the 2-day serum as a reference standard. Most of the radioiodine in each sample sedimented in a single, slowly moving zone with an approximate sedimentation coefficient of 5 or 6 S (Chart 1). The iodoprotein and the thyroxine-serum protein complexes, therefore, could not be distinguished from each other. A small peak of 131I was observed in the 19 S (thyroglobulin) region and amounted to 2% of the total in the 4-day serum. In the case of whole serum, most of the 131I was found to coincide with the albumin and γ-globulin precipitin arcs, but there were from 1 to 3 additional, fainter arcs seen in the α- and β-globulin areas. In the case of the iodoprotein fractions, radioactivity was observed only in the albumin and γ-globulin precipitin arcs. The increased radioactivity evident in the leading portion of the albumin arc was seen in all cases, but this was not always so accentuated as in Fig. 1. Also shown in Fig. 1 are the results of simple zone electrophoresis of the iodoprotein fraction on the agar plates. Most of the 131I is seen in the albumin zone, but the β- and γ-globulin zones are also clearly radioactive despite their migration in a direction opposite from that of albumin. Although not visible in the reproduction, the leading edge of the albumin zone in the autoradiograph of Pattern E contained relatively more radioactivity than the trailing edge.

Rat Thyroid Tumors

The distribution of 131I in rat tumors, thyroid, and plasma at 48 hr after the dose is reported in Table 1. Tumors of Line 1-1c, 1-1c slow, and 1-8 were active in concentrating iodine, and the plasma radioiodine in these animals was higher than in lines 16-6 and 1-2, in which the tumors showed little or no activity (the tumor:plasma ratio was less than 1). The data for the latter group are given to provide a comparison for thyroid and plasma radioiodine levels in rats with active and relatively inactive tumors. Subsequent studies are limited to the 3 active tumor lines.

Serum Iodoproteins. The results of column chromatographic analysis of serum samples from the rats with active tumors are presented in Table 3. In each case, the major component (other than iodide) was in the iodoprotein fraction, amounting to 80% of the organic iodine in Line 1-1c, 81% in Line 1-1c, slow, and 94% in Line 1-8. The serum iodoprotein, isolated by chromatography on Dowex 1-X2 columns, was analyzed by density gradient ultracentrifugation. The results, which are illustrated in the bottom portions of Charts 3 and 4, were similar in each of the active tumor lines. Most of the radioactivity was in a single peak with a sedimentation coefficient of 6 S or 7 S. The radioiodine was more or less coincident with total protein distribution.

Immunoprecipitation studies were carried out on the serum iodoprotein fractions in rats bearing tumor Lines 1-1c, slow, and 1-8. The results are illustrated in Charts 5 and 6. In tumor Line 1-1c, slow, there was almost complete precipitation of serum iodoprotein by antiserum against normal rat serum. In Line 1-8, the precipitation was somewhat less complete. The specificity of

\[ \text{TABLE 3} \]

<table>
<thead>
<tr>
<th>Tumor Line</th>
<th>% of total 131I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iodoprotein</td>
</tr>
<tr>
<td>1-1c</td>
<td>51</td>
</tr>
<tr>
<td>1-1c, slow</td>
<td>36</td>
</tr>
<tr>
<td>1-8</td>
<td>51</td>
</tr>
</tbody>
</table>

* All samples taken at 48 hr after administration of 131I.
Iodinated Serum Proteins

**Chart 3.** Density gradient ultracentrifugation of thyroid tumor extract, and of serum iodoprotein isolated by Dowex 1-X2 chromatography, in rats bearing Tumor 1-1e.

**Chart 4.** Density gradient ultracentrifugation of thyroid tumor extract, and of serum iodoprotein isolated by Dowex 1-X2 chromatography, in rats bearing Tumor 1-8. The zone labeled 19S indicates the location of rat thyroglobulin in a control tube during the same run.

**Chart 5.** Immunoprecipitation of serum iodoprotein (obtained by Dowex 1-X2 chromatography) and tumor 6S iodoprotein (isolated by density gradient centrifugation) in rats bearing Tumor 1-1e, slow. Rabbit antiserum against normal rat serum was used.

The precipitin reaction was investigated in the case of tumor Line 1-1e, slow, and the results are given in Table 4. The precipitation of the serum iodoprotein was prevented by the presence of an excess of normal rat serum.

Immunoelectrophoretic analyses of the serum iodoprotein fractions are shown in Fig. 2. The stained patterns indicate that these fractions contained many, if not all, of the serum proteins.
The data presented here provide strong evidence for the occurrence of iodinated serum proteins in the serum of patients and animals with functioning thyroid tumors. Iodinated albumin...
comprised most of this serum iodoprotein and, indeed, accounted for almost all of it in the patient studied, but electrophoretic and immuno-electrophoretic analyses indicated that various iodinated serum globulins also were present. In rats bearing Tumor 1-8, the serum iodoprotein was not completely precipitated by anti-rat serum, but in the other cases precipitation was more extensive. The specificity of the precipitin reactions was indicated by the fact that they could be inhibited by normal serum proteins.

In the thyroid tumors, which were studied only in rats, a relatively small proportion of the iodoprotein could be identified as iodinated serum proteins. Even when attention was limited to the slowly sedimenting proteins, only partial precipitation could be obtained with anti-rat serum. Nevertheless, the presence of iodinated serum albumin and serum globulins appeared to be unequivocal in tumors of Line 1-1c and 1-1c, slow.

These iodinated serum proteins presumably arise from the fact that serum proteins come in contact with the iodinating system in the thyroid gland. Although they can be found in normal subjects, their quantity is increased in a variety of thyroid diseases, including tumors, which probably indicates increased vascularity may also be a factor, since many of the diseases in which iodinated serum proteins are found are characterized by thyroid hyperplasia.

The nature of the slowly sedimenting iodoproteins that were not precipitated by antisera against serum proteins was not established. This type of material was especially evident in tumors in Line 1-8, in which it comprised most of the tumor radiiodine and also appeared to be present in the blood of these animals. It is possible that these are iodinated serum proteins that have been altered in such a way that they no longer form a specific precipitate. It is also possible that they are entirely unrelated to the serum proteins and that they are abnormal iodoproteins produced by the tumor. The known subunits of thyroglobulin would be expected to occur as discretely sedimenting zones in the 6 S and 12 S regions. Further studies on these unusual iodoproteins in thyroid tumors will be of interest.

References

FIG. 1. Immunoelectrophoresis and agar gel electrophoresis of serum iodoprotein from patient D.P., 3 days after $^{131}$I therapy. The wells labeled C and E contained iodoprotein obtained by Dowex 1-X2 chromatography of the patient's serum. Wells A and D contained normal human serum. The trough labeled B contained horse antiserum against whole human serum.
FIG. 2. Immunoelectrophoresis of serum iodoprotein (obtained by Dowex 1-X2 chromatography) in rats bearing thyroid tumors. Well B: tumor Line 1-1c; Well C: Line 1-8; Well E: Line 1-1c, slow. Troughs A and D contained goat antiserum against normal rat serum.

FIG. 3. Immunoelectrophoresis of rat tumor extract and tumor 6 S or 9 S iodoprotein (isolated by density gradient centrifugation). Well A: Line 1-1c, 6 S iodoprotein; Well C: Line 1-1e, extract; Well D: Line 1-1c, slow, 6 S iodoprotein plus normal rat serum; Well F: Line 1-1c, slow, 6 S iodoprotein; Well G: Line 1-8, 9 S iodoprotein plus normal rat serum; Well I: Line 1-8, 9 S iodoprotein. Trough B: goat antiserum against normal rat serum; Troughs E and H: rabbit antiserum against normal rat serum. In those cases where normal rat serum was added to the iodoprotein, the proportion was 1 part (by volume) of serum to 2 parts of iodoprotein solution.
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