Interaction of Thyroid Peroxidase with Concanavalin A Covalently Coupled to Agarose*

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We have investigated the interaction between concanavalin A-agarose (Con A-agarose) and thyroid peroxidase, an integral membrane protein found in the 105,000 × g, 1-h particulate fraction of thyroid tissue. An intact form of porcine thyroid peroxidase was obtained by solubilization with the nonionic detergent Triton X-100 and two fragmented, hydrophilic forms of the enzyme were prepared by trypsin treatment of the membrane. The three types of thyroid peroxidase bind to Con A-agarose and can be eluted with α-methyl-D-mannoside. The α-methyl-D-mannoside eluate of the most purified thyroid peroxidase preparation has been analyzed by polyacrylamide gel electrophoresis. Peroxidase activity corresponds with a glycoprotein band.

The binding of thyroid peroxidase to Con A-agarose can be inhibited by sugars in the following order: α-methyl-D-mannoside > D-mannose > α-methyl-D-glucoside > D-glucose > D-galactose. This order of specificity is typical of Con A-protein interactions. Furthermore, inactivation of the carbohydrate binding site of Con A by demetallization greatly reduces the extent of thyroid peroxidase binding. Reactivation of the carbohydrate binding site by the addition of Ca2+ and Mn2+ to demetallized Con A-agarose restores thyroid peroxidase binding.

These and other experiments suggest that thyroid peroxidase is, like several other peroxidases, a glycoprotein. In addition, the interaction between thyroid peroxidase and Con A-agarose may provide a new purification tool for thyroid peroxidase.

Thyroid peroxidase is an integral membrane enzyme (1) which plays a key role in the biosynthesis of thyroid hormones because of its ability to catalyze the iodination of tyrosine residues in thyroglobulin. The properties of thyroid peroxidase and the mechanism of iodination have not been clearly elucidated.

The most widely used procedure for solubilizing thyroid peroxidase has been proteolysis (2–8). However, we have shown that trypsin solubilization is likely to alter the molecular size of the native peroxidase (9). In order to study the intact enzyme, we have developed nonproteolytic methods of solubilization (10–12) and have recently reported on the solubilization of thyroid peroxidase by nonionic detergents (1).

The purification of intact thyroid peroxidase to homogeneity has not been achieved to date, but one way to obtain information about the properties of the intact peroxidase is to study its interaction with molecules that have specific, well-characterized binding properties and to compare these observations with similar studies conducted with the more purified, fragmented form of thyroid peroxidase. Since several other peroxidases, i.e. horseradish and chloro- and lactoperoxidase (13, 14), are glycoproteins, and since Taurog has reported that the partially purified, fragmented form of thyroid peroxidase gives a positive orcinol test (7), we decided to study the interaction of intact and fragmented thyroid peroxidase with Con A, a protein that specifically binds saccharides and glycoproteins (15–18). Here we present new and more direct evidence on the glycoprotein nature of thyroid peroxidase.

**EXPERIMENTAL PROCEDURES**

Preparation of Triton X-100-Solubilized Thyroid Peroxidase — The 105,000 × g, 1-h particulate fraction from frozen porcine thyroids (Swift & Co., Chicago, III.) was prepared as previously described (15) and stored at -20°. In order to release occluded cytoplasmic proteins, the particulate fraction was suspended in 0.1 mM KI, mixed for 2 to 4 h on a Dubnoff shaker at 5°, and centrifuged at 105,000 × g, 1 hr, 5°. Solubilization of thyroid peroxidase by 0.1% Triton X-100 and at a particular protein concentration of 1.5 mg/ml was conducted as previously described (1). The supernatant, which contained about 65% of the thyroid peroxidase activity, was concentrated by ultrafiltration. The specific activity of the Triton X-100 extract ranged between 1.2 and 3.6 μmol of oxidized guaiacol/min/mg of protein.

Preparation of Trypsin-solubilized Thyroid Peroxidase — Two types of preparations have been utilized. In one case, thyroid peroxidase was prepared by the method of Pommier et al. (19) which involves trypsin treatment of the membrane followed by digitonin extraction. Because of the reported instability of the enzyme after the final purification step (19), this step was omitted. The specific activity of the preparation was 79 μmol of oxidized guaiacol/min/mg of protein. In the second case, a modification of the procedures described by Taurog and co-workers (4, 6, 7) and Hosoya and Morrison (3) was employed. With the exception of the trypsin digestion, all steps were conducted at 4°. The 105,000 × g, 1-h pellet containing 3.2 g of protein was washed in 0.1 mM KI, as described above, and the pellet was suspended in 10 mM Tris/HC1 (pH 8), 5 mM CaCl2, 0.1 mM KI to a
protein concentration of 5.4 mg/ml. Thyroglobin (5 mg/ml of particulate protein) was added and the suspension was incubated at 37°C for 1 h. Protamine was terminated by adding a 2.5-fold excess of soybean trypsin inhibitor and a supernatant was obtained by centrifugation at 105,000 x g overnight in a Beckman 50 Ti rotor (5°C). Artificial thyroid peroxidase was solubilized by this procedure with 20% and 10% in the first and second digestions, respectively. Two supernatants were combined and chromatographed on a DEAE-cellulose (Whatman, DE52) column (2.5 x 50 cm) equilibrated in 15 mM Tris/HC1 (pH 7.4), 25 mM KC1, 0.1 mM KC1. After sample application, the column was washed with 300 ml of equilibration buffer and then developed with a 500-ml linear KC1 gradient. The mixing chamber initially contained 250 ml of equilibration buffer and the reservoir contained 250 ml of 0.25 M KC1, 10 mM Tris/HC1 (pH 7.4), 0.1 mM KC1. Peroxidase activity eluted in the range of 0.15 M KC1. After concentration by ultrafiltration, the peroxidase fraction was applied to a Sephacryl 4B column (1.5 x 90 cm) equilibrated in 10 mM Tris/HC1 (pH 7.4), 0.1 mM KC1. The peak peroxidase activity eluted at 106 ml; the void volume was 48 ml, as determined with blue dextran. After concentration by ultrafiltration, the peroxidase fraction was applied to a Sephacryl G-200 column (2.5 x 95 cm) equilibrated in 10 mM Tris/HC1 (pH 7.4), 0.1 mM KC1. The peak peroxidase activity eluted at 250 ml; the void volume was 150 ml, as determined with blue dextran. The peroxidase fraction was concentrated by ultrafiltration to a protein concentration of 5.4 mg/ml. The final preparation was purified about 25-fold over the 105,000 x g, 1 h, particulate fraction and had a specific affinity of 65 μmol of oxidized guaiacol/min/mg of protein. The trypsin-solubilized peroxidase prepared by Hossoya and Morrison (3) had a specific activity of 48.9 μmol of oxidized guaiacol/min/mg of protein.

Preparation of Con A-Agarose and Demethylized Con A-Agarose - Sepharose 4B was activated by reaction with cyanogen bromide (20 mg of CNBr/ml of settled agarose) (20) and washed with 0.1 M NaHCO3, pH 7.2 before addition of 2 mg of Con A/ml of settled agarose in 0.1 M NaHCO3, pH 7.2, and 0.15 M NaCl. After gentle mixing at 25°C for 48 h, the product was washed with 0.1 M NaHCO3, H2O, and 1 M NaCl. Remaining active groups were blocked by treatment with 1 M glycine, pH 9, for 2 h (22). The Con A-agarose was finally washed with 0.15 M NaCl and stored at 4°C. The efficiency of the coupling reaction, as judged by the extraction of the washings at 280 nm, ranged from 62 to 80% for six batches of Con A-agarose. For a binding control, "activated" agarose was prepared by removing a portion of the cyanogen bromide-treated agarose before addition of Con A. This sample was then washed in exactly the same manner as the Con A-agarose. Demethylized Con A-agarose was prepared by treating Con A-agarose with 0.1 N HCl for 30 min, followed by exhaustive dialysis against H2O. Sumner and Howell (23) first described the demethylation of Con A in 1936, and more recently Naib and Levitizki (24) reported the details of the procedure that was followed here.

Conditions of Binding and Elution - These are described in the legends to the tables and figures.

Preparation of Immobilized Anti-porcine Thyroglobulin - Thyroglobulin, the major glycoprotein in thyroid tissue, is found in many thyroid peroxidase preparations. In order to remove thyroglobulin from the peroxidase preparations, anti-porcine thyroglobulin was prepared and immobilized on glass beads. The use of immobilized anti-thyroglobulin was necessary since thyroglobulin was subsequently measured quantitatively by radioimmunoassay. Use of free antiserum to remove thyroglobulin would result in a peroxidase preparation contaminated with anti-thyroglobulin which would interfere with the radioimmunoassay of thyroglobulin. Anti-porcine antiserum to remove thyroglobulin would result in a peroxidase preparation contaminated with anti-thyroglobulin which would interfere with the radioimmunoassay of thyroglobulin. Therefore, it is necessary to remove thyroglobulin from the Triton X-100 peroxidase fraction before proceeding with the radioimmunoassay of thyroglobulin. This was accomplished by centrifugation, the pellets were counted in a Packard y spectrometer. These experiments showed that 4 mg of anti-thyroglobulin-CGP in a 0.5% (w/v) suspension absorbed a maximum of 85% of the thyroglobulin from a Triton X-100 extract containing 0.1 mg of protein. Thyroglobulin automation was quantitatively determined by double immunodiffusion against anti-porcine thyroglobulin. No precipitin line was observed between the immunoadsorbed Triton X-100 extract and anti-porcine thyroglobulin, whereas a strong line developed between an untreated Triton X-100 extract and the antisera. Thyroglobulin was quantitatively measured by radioimmunoassay for porcine thyroglobulin, using a procedure similar to the radioimmunoassay described by Van Herle et al. (26), except that porcine antigen and anti-porcine thyroglobulin were substituted for human thyroglobulin and anti-thyroglobulin. Assays of the Triton X-100 extract before and after immunoadsorption indicated that 95% of the thyroglobulin was adsorbed from the Triton X-100 extract by anti-thyroglobulin-CGP. (Use of the radioimmunoassay also showed that no detectable anti-thyroglobulin leaked off the glass beads since the supernatant from the anti-Thyroglobulin-CGP-immunoadsorbed sample did not bind 125I-thyroglobulin.) The immunoadsorbed Triton X-100 extract is referred to as the "thyroglobulin-depleted thyroid peroxidase."
Interaction of Thyroid Peroxidase with Concanaualin A

Dilute acid destroys the ability of Con A to interact with the carbohydrate binding site. The first supernatant contained 85% of the recovered peroxidase activity. The supernatants were assayed for peroxidase activity and for protein, as described under "Experimental Procedures." The values in the table are the total of the first supernatant and the wash. The first supernatant contained 85% of the recovered peroxidase activity.

| Experiment | Thyroid peroxidase activity | Protein |
|------------|----------------------------|---------|
| Agarose    | 2.03                       | 1.48    |
| "Activated" agarose | 2.92   | 1.53    |
| Con A-agarose | 0.96   | 1.06    |
| Con A-agarose + α-methyl-α-mannoside | 2.00  | 1.16    |

thereby suggesting that thyroid peroxidase and α-methyl-α-mannoside compete for the carbohydrate binding site on Con A.

Sugar Inhibition of Interaction of Thyroid Peroxidase with Con A-Agarose – As shown in Table II, the binding of thyroid peroxidase to Con A-agarose can be specifically inhibited by sugars. α-Methyl-α-mannoside (10 mg/ml) inhibits binding by 50%; d-mannose and α-methyl-d-glucoside are also effective inhibitors, d-glucose is less effective, and d-galactose has little effect on the binding of thyroid peroxidase. Goldstein and co-workers (15) studied the specificity of the carbohydrate binding site of Con A by investigating the ability of various sugars to inhibit the dextran-Con A precipitation reaction. The sugars that were tested in our study inhibit the interaction between thyroid peroxidase and Con A in the same order of specificity as in the dextran-Con A interaction investigated by Goldstein et al. (15). We interpret this high degree of specificity as further evidence that thyroid peroxidase binds to the carbohydrate binding site of Con A. Katzen and Soderman (34) found a similar order of inhibition by sugars for the interaction of adipocyte receptors with the carbohydrate binding sites of Con A-agarose.

Studies with Demetallized Con A-Agarose – In 1936, Sumer and Howell (23) reported on the role of divalent metal ions in the reversible inactivation of jack bean hemagglutinin. Subsequent studies (24, 35) have confirmed the observation that removal of divalent cations from Con A by treatment with dilute acid destroys the ability of Con A to interact with polysaccharides and that the addition of Ca²⁺ and Mn²⁺ can restore carbohydrate binding. However, demetallization does not destroy the ability of Con A to bind hydrophobic molecules such as the dye rose bengal. As shown in Fig. 1, when increasing amounts of thyroid peroxidase are added to a prepa-

R. H. Glew, personal communication.
binds is probably due to incomplete demetalization of the Con A-agarose.) However, when the carbohydrate binding sites are restored by the addition of Ca²⁺ and Mn²⁺ to demetalized Con A-agarose, thyroid peroxidase binding increases dramatically and reaches a saturating level, much greater than that obtained in the absence of Ca²⁺ and Mn²⁺.

**Elution of Thyroid Peroxidase from Con A-Agarose with α-Methyl-D-mannoside** — Table III shows the ability of α-methyl-D-mannoside to release detergent-solubilized thyroid peroxidase from Con A-agarose. Approximately 60% of the thyroid peroxidase activity was recovered in the combined supernatants and most of the recovered activity was found in the first α-methyl-D-mannoside supernatant. A control experiment showed that α-methyl-D-mannoside is essential for thyroid peroxidase release since only 4% of the applied activity was eluted in the absence of α-methyl-D-mannoside.

Experiments conducted over a period of 1 year indicate that the amount of thyroid peroxidase released from Con A-agarose by α-methyl-D-mannoside can vary from 30 to 95%, depending on the batch of Con A-agarose and on the thyroids used as starting material for the preparation of the thyroid particulate fraction. From the same batches of Con A-agarose, we found that the elution of a known glycoprotein, porcine thyroglobulin (Sigma, type II), varied from 30 to 60%. The consistency of glycoprotein elution from lectin-agarose systems has not been discussed frequently, although a variable elution of α-glutamyltransferase by mannose has been reported (36), and yields ranging from 30% (37) to 75% (38) of electric eel acetylcholinesterase have been described. The recovery of thyroid peroxidase from Con A-agarose compares favorably with the yields obtained for other proteins from lectin-agarose systems. For example, 40% of human luteinizing hormone and 85% of human chorionic gonadotropin (39), 25% of human plasma lipase (40), 60% of a-fetoprotein (41), and 90% of rhodopsin (29) have been recovered from Con A-agarose.

The fractions described in Table III were subjected to polyacrylamide gel electrophoresis in the presence of Na dodecyl SO₄ and the results are shown in Fig. 2. It is not possible to identify thyroid peroxidase in these electrophoresis gels because the peroxidase is inactivated by diithiothreitol and Na dodecyl SO₄. The fraction eluted with α-methyl-D-mannoside contains three readily visible glycoprotein bands and several faint bands which are not visible in the photograph. The protein bands in the α-methyl-D-mannoside eluted fractions that stain with Coomassie blue but do not stain with the PAS reagent may represent glycoproteins which are low in sialic acid content. It is generally accepted that sialic acid-rich glycoproteins readily stain with PAS (42, 43). However, not all glycoproteins react with the PAS reagent. Adair and Kornfeld (44) have reported on a glycoprotein receptor from human erythrocytes for *Ricinus communis* agglutinin which does not stain with PAS reagent. This glycoprotein is rich in N-acetylglucosamine but almost devoid of sialic acid.

In the fraction which does not bind to Con A, a strong PAS band is present close to the tracking dye. The position of this fast moving band is similar to that reported by Lenard (45) and Fairbanks et al. (32) and may be a glycolipid. This component may account for most of the sugars in the fraction that does not bind to Con A (Table III). Some glycoproteins, which do not have an affinity for Con A-agarose and which do not react with the PAS stain, may also be present in this fraction.

**Effect of pH on α-Methyl-D-mannoside-dependent Release of Thyroid Peroxidase** — As shown in Fig. 3, the optimum pH for the release of thyroid peroxidase with α-methyl-D-mannoside is about 9. Under these conditions, 60% or more of the peroxidase is released. In the absence of α-methyl-D-mannoside only 6% or less of the peroxidase is eluted at pH 8.9, thereby indicating that the increase in pH is not sufficient to release

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**Table III**

Release of thyroid peroxidase activity, protein, and sugar from Con A-agarose by α-methyl-D-mannoside

| Fraction                  | Thyroid peroxidase activity | Protein  | Sugar  |
|---------------------------|----------------------------|----------|--------|
|                           | guaiacol units             | mg       | μg     |
| Applied to Con A-agarose  | 2.82                      | 2.60     | 360    |
| Not bound                 | 0                         | 1.10     | 170    |
| α-Methyl-D-mannoside eluate | 1.78                      | 1.06     | 61     |

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**Fig. 2.** Na dodecyl-SO₄-polyacrylamide gel electrophoresis of the samples listed in Table III. The gels were stained for protein with Coomassie blue (CB) and for carbohydrate with periodic acid-Schiff reagent (PAS). The arrows indicate the positions of the main carbohydrate-positive components in the "not bound" fraction and the α-methyl-D-mannoside (α-MM) eluate.
Interaction of Thyroid Peroxidase with Concanavalin A

The decrease in the activity curve above pH 9.5 may be due in part to the slight decrease in activity of peroxidase in alkaline medium (10) rather than a decrease in amount of peroxidase eluted above pH 9.

Interaction of Con A-Agarose with Thyroid Peroxidase

Preparation Treated with Immobilized Anti-thyroglobulin—Thyroglobulin, the major glycoprotein in thyroid tissue, is found in many peroxidase preparations. As shown in Table IV, when 85% of the thyroglobulin is removed from the detergent-solubilized peroxidase preparation by immunoprecipitation, none of the peroxidase activity is lost. Furthermore, the thyroglobulin-depleted peroxidase binds to and elutes from Con A-agarose as efficiently as the untreated peroxidase.

Interaction of Two Highly Purified Thyroid Peroxidase Preparations with Con A-Agarose—A fragmented, hydrophilic form of thyroid peroxidase (8, 7, 19) can be obtained in a state of greater purity than the detergent-solubilized, intact peroxidase. For the experiments shown in Table V, fragmented thyroid peroxidase was prepared by the method of Taureg and associates (6, 7), with the modifications given under "Experimental Procedures." This preparation is at least 25% pure, as judged by a peroxidase activity stain and a protein stain of polyacrylamide electrophoresis gels (6). As shown in Table V, this fragmented form of thyroid peroxidase also binds to Con A-agarose and can be eluted with α-methyl-D-mannoside (Experiments 2 and 3). Furthermore, when α-methyl-D-mannoside is mixed with this form of thyroid peroxidase prior to addition to Con A-agarose, peroxidase binding to Con A is greatly reduced (Experiment 4).

The interaction between Con A and thyroid peroxidase pre-

![Fig. 3. The effect of pH on the α-methyl-D-mannoside-dependent release of thyroid peroxidase from Con A-agarose. One milliliter (settled volume) portions of Con A-agarose were equilibrated in 10 mM phosphate buffer (pH 6.0), 0.1% Triton X-100, 1 mM CaCl₂, 1 mM MnCl₂, 0.1 mM KI (equilibrating buffer) as described in Table III. Three hundred microliters of a Triton X-100 extract were mixed with 2.2 ml of equilibrating buffer and added to each tube containing washed Con A-agarose. The suspensions were mixed at 5°C for 25 h and supernatants were obtained by centrifugation at 2000 rpm in a Sorvall HI-4, swinging bucket rotor for 10 min, 5°C. Each gel was washed twice with 2.5 ml of 10 mM phosphate buffer (pH 6.0), 0.1% Triton X-100, 0.1 mM KI. The peroxidase activity in the first supernatants ranged from 2.3 to 2.7% of the applied activity and no peroxidase activity was detected in the washes. The gels were then mixed with 2.5 ml of α-methyl-D-mannoside (100 mg/ml) in 0.1 M buffer (pH as indicated in abscissa), 0.1% Triton X-100, 0.1 mM KI at 5°C, overnight. Supernatants were obtained and analyzed for peroxidase activity (O-O) and protein (O- - -O) (after dialysis against 1 mM phosphate buffer, pH 7.4). The buffers employed were: pH 6.0, phosphate; pH 7.4, 8.2, 8.9, Tris/HCl; pH 9.3, 9.8, 10.3, carbonate/bicarbonate.

![Table IV. Immunoadsorption of thyroglobulin from thyroid peroxidase preparation and binding and elution properties of immunoadsorbed preparation on Con A-agarose

For Sample 1, 270 μl of a Triton X-100 extract were added to 3.0 ml of 0.1% Triton X-100, 10 mM Tris (pH 7.4), 0.1 mM KI. For Sample 2, 270 μl of the same Triton X-100 extract were added to 2.75 ml of a 0.5% (w/v) immobilized anti-porcine thyroglobulin mixture that had previously been exhaustively washed with the 0.1% Triton X-100, 10 mM Tris (pH 7.4), 0.1 mM KI buffer to remove bovine serum albumin and sodium azide. After intermittent mixing for 3 h at room temperature, both samples were centrifuged at 2200 × g for 10 min, and 2 ml of each supernatant (initial sample) were added to separate tubes containing 1 ml (settled volume) of Con A-agarose and 1 ml of 10 mM phosphate (pH 6.0), 0.1% Triton X-100, 0.1 mM KI, 0.1 mM CaCl₂, and 1 mM MnCl₂. The Con A-agarose had previously been equilibrated as described in Table III. The suspensions were mixed for 1.5 h at 4°C and supernatants were obtained as described in Table III. The gels were washed with 3 ml of the α-methyl-D-mannoside solution but no further peroxidase activity was obtained in the subsequent supernatant.

| Sample                  | Thyroglobulin | Thyroid peroxidase activity |
|-------------------------|--------------|-----------------------------|
|                         | μg           | Initial | Bound | Eluted |
| 1. Thyroid peroxidase   | 252          | 0.51    | 0.46   | 0.17   |
| 2. Thyroglobulin-depleted thyroid peroxidase | 40 | 0.55 | 0.49 | 0.17 |

Interaction of trypsin-solubilized, highly purified thyroid peroxidase with Con A-agarose

Con A-agarose (0.5 ml settled volume) was washed three times with 2.5 ml of 10 mM phosphate buffer (pH 6.0), 1 mM CaCl₂, 0.1 mM KI (equilibrating buffer). Trypsin-solubilized, highly purified thyroid peroxidase was diluted about 1-fold with equilibrating buffer (for Experiments 1 and 2) or with equilibrating buffer containing 100 mg/ml of α-methyl-D-mannoside (for Experiment 4). The samples were added to the equilibrated Con A-agarose and the suspensions were mixed for 1.5 h at 4°C. Supernatants were obtained as described in Table I and the packed gels were washed twice with 10 mM phosphate buffer (pH 6.0), 0.1 mM KI. For Experiment 3, the gel from Experiment 2 was washed with 2.5 ml of 100 mg/ml of α-methyl-D-mannoside in 0.1 M Tris/HCl (pH 8.9), 0.1% Triton X-100, 0.1 mM KI were added to each gel and the suspensions were mixed overnight at 4°C. A supernatant was obtained by centrifugation as described in Table III. The gels were washed with 3 ml of the α-methyl-D-mannoside solution but no further peroxidase activity was obtained in the subsequent supernatant.

| Experiment | Materials recovered in supernatants
|------------|--------------------------------|
|            | Thyroid peroxidase | Protein |
| 1. Starting thyroid peroxidase sample | 8.00 | 0.21 |
| 2. Add thyroid peroxidase to Con A-agarose | 1.06 | 0.075 |
| 3. Treat Con A-agarose from 2 above with α-methyl-D-mannoside | 7.30 | 0.133 |
| 4. Mix thyroid peroxidase and α-methyl-D-mannoside, then add to Con A-agarose | 7.51 | 0.098 |
judged by electrophoretic techniques (19). Results similar to those presented in Table V have been obtained with this preparation. In addition, the o-methyl-n-mannoside-eluted fraction corresponds with a glycoprotein band.

This evidence strongly suggests that thyroid peroxidase is a glycoprotein which binds to the carbohydrate binding site of Con A. However, alternative explanations for the interaction of thyroid peroxidase with Con A have been considered since the binding of membrane components and glycoproteins to Con A may involve more than sugar-Con A interactions (18, 46-49) and since the thyroid peroxidase is not available in a homogeneous state. One type of noncarbohydrate interaction with Con A that has been reported involves hydrophobic binding (47, 50-52). However, the following observations indicate that hydrophobic interactions do not play a major role in the binding of thyroid peroxidase to Con A-agarose. First, demetallization of Con A greatly reduces the binding of thyroid peroxidase (Fig. 1). Demetallization destroys the carbohydrate binding site of Con A (23, 24, 35), but does not alter the ability of Con A to bind hydrophobic molecules. Addition of Ca2+ and Mn2+ reactivates the carbohydrate site of Con A and restores the binding of thyroid peroxidase (Fig. 1). Second, the fragmented form of thyroid peroxidase, which does not exhibit hydrophobic properties, binds to and elutes from Con A-agarose as effectively as the intact form of thyroid peroxidase. This indicates that the hydrophobic portion of the intact peroxidase is not responsible for the binding of thyroid peroxidase to Con A. Third, the Triton X-100 micelles present in the experiments described here for intact thyroid peroxidase would be expected to block the hydrophobic binding site of Con A. Fourth, we were not able to improve the recovery of intact thyroid peroxidase from Con A-agarose by use of ethylene glycol, a polarity reducing solvent which was effective in increasing the yield of human interferon from ConA-agarose (52). This protein binds to Con A-agarose both by hydrophobic interaction and carbohydrate recognition.

It has also been suggested that protein-protein interactions, i.e. between Con A and adsorbed proteins, which are mediated by electrostatic forces, may stabilize the glycoprotein-Con A complex (18, 53). The combined requirement of sugar and elevated pH for maximum release of thyroid peroxidase from Con A-agarose may reflect such interactions (Fig. 3). Bishayee and Bachhawat (54) also reported a pH-dependent release of a lysosomal glycoprotein, arylsulfatase A, from a Con A-glycoprotein precipitate. However, this type of protein-protein interaction cannot solely account for the interaction of thyroid peroxidase and Con A-agarose because of the evidence summarized in the first paragraph under "Discussion." and the fact that, in the absence of sugar, only about 5% of the peroxidase can be eluted at pH 9.

Finally, since the peroxidase is not pure, we have considered the possibility that the interaction between Con A and thyroid peroxidase could be mediated by a glycoprotein contaminant in the peroxidase preparations. Thyroglobulin is the major glycoprotein contaminant of crude thyroid peroxidase preparations. A thyroglobulin-thyroid peroxidase complex, if pres-

**DISCUSSION**

The data presented here can be summarized as follows: (a) thyroid peroxidase binds to Con A-agarose and the binding is inhibited by monosaccharides in the same general order of specificity as observed in the competition between monosaccharides and dextran for the carbohydrate binding site of Con A; (b) the binding of thyroid peroxidase to Con A-agarose can be reversed by the addition of o-methyl-D-mannoside to the

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**Fig. 4. Polyacrylamide gel electrophoresis of thyroid peroxidase eluted from Con A-agarose with o-methyl-D-mannoside.** Thyroid peroxidase as prepared by the method of Nunez and co-workers (19) was applied to Con A-agarose and eluted with o-methyl-D-mannoside as described in Table V. The eluted fraction was concentrated and the sugar removed by vacuum dialysis against 5 mM phosphate buffer (pH 7.4) containing 0.1 mM KI. Samples (35 μg of protein/gel) were then applied to polyacrylamide gels and electrophoresis was conducted as described under "Experimental Procedures." Individual gels were stained for (a) thyroid peroxidase (TPO) activity by means of the guaiacol/H2O2 assay reagent; (b) for carbohydrate by means of the PAS reagent; and (c) for protein with Coomassie blue (CB). The black lines near the bottom of the gels indicate the position of the tracking dye. The arrow denotes the correspondence between the peroxidase activity band, the PAS band, and the major protein band. The peroxidase activity band developed within 10 min of H2O2 addition.
could conceivably bind to Con A-agarose by means of the glycolic moieties of thyroglobulin. However, under the conditions employed here, it does not appear that a thyroglobulin-peroxidase or a thyroglobulin subunit-peroxidase complex is present in solution. Removal of 85% of the thyroglobulin by immunoprecipitation from the intact peroxidase preparation does not decrease the peroxidase activity in solution and does not alter the ability of intact thyroid peroxidase to bind to or elute from Con A-agarose (Table IV). Furthermore, experiments designed to measure the physicochemical properties of the intact and fragmented thyroid peroxidase indicate that the molecular weight of the detergent-extracted peroxidase is about 135,000, as determined by the method of Clarke (55). A much higher value for the molecular weight would be expected for a thyroglobulin (660,000) peroxidase complex or a thyroglobulin subunit (180,000). peroxidase complex.

Values for the partial specific volume, \( \bar{\nu} \), of intact and fragmented thyroid peroxidase have been obtained by the method of Clarke (55). The experimentally determined \( \bar{\nu} \) for fragmented thyroid peroxidase prepared by the method of Taurog is 0.68 ml/g and that for the intact enzyme is 0.72 ml/g. The higher value obtained for intact peroxidase reflects the binding of about 0.2 g of Triton X-100 per g of detergent-solubilized enzyme. A \( \bar{\nu} \) of 0.68 ml/g for thyroid peroxidase suggests that this protein contains significant glycolic moieties (56). If one assumes that the \( \bar{\nu} \) of a "typical" protein devoid of carbohydrates is about 0.73 ml/g and the \( \bar{\nu} \) of sugar residues is about 0.60 ml/g (56), one can calculate that the carbohydrate composition of thyroid peroxidase is about 35%.

In addition, the electrophoresis data presented here (Fig. 4) also indicates that thyroid peroxidase is a glycoprotein. Analyses of the fragmented form of thyroid peroxidase on polyacrylamide gels after electrophoresis in the absence of Na dodecyl-SO4 show that the peroxidase activity band corresponds with the glycoprotein band.

In conclusion, we believe that thyroid peroxidase is a glycoprotein since (a) the peroxidase activity corresponds to a carbohydrate stain, (b) the \( \bar{\nu} \) is characteristic of glycoproteins (56), and (c) three thyroid peroxidase preparations solubilized and partially purified by different procedures bind to Con A-agarose and can be eluted with \( \alpha \)-methyl-D-mannoside. However, because the peroxidase is not pure, we have considered the possibility that the binding of thyroid peroxidase to Con A could be mediated by a glycoprotein contaminant in the preparation. This possibility seems unlikely, but it cannot be completely excluded until a homogeneous preparation of thyroid peroxidase is available. We hope that these studies will help to achieve this goal by providing a new tool for the purification of this and other peroxidases.

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REFERENCES

1. Neary, J. T., Davidson, B., Armstrong, A., Streut, H. V., Soodak, M., and Maloof, F. (1976) J. Biol. Chem. 251, 2525-2529
2. DeGroot, L. J., Thompson, J. E., and Dunn, A. D. (1965) Endocrinology 76, 632-645
3. Hosoya, T., and Morrison, M. (1967) J. Biol. Chem. 242, 2828-2836
4. Coval, M. L., and Taurog, A. (1967) J. Biol. Chem. 242, 5510-5523
5. Ljunggren, J.-G., and Akeson, A. (1968). Arch. Biochem. Biophys. 127, 346-353
6. Taurog, A., Lothrup, M. L., and Estabrook, R. W. (1970) Arch. Biochem. Biophys. 139, 221-229
7. Taurog, A. (1970) Recent Progr. Horm. Res. 26, 189-247
8. Danner, D. J., and Morrison, M. (1971) Biochim. Biophys. Acta 235, 44-51
9. Neary, J. T., Armstrong, A., Davidson, B., Maloof, F., and Soodak, M. (1975) Biochim. Biophys. Acta 379, 262-270
10. Neary, J. T., Davidson, B., Maloof, F., and Soodak, M. (1973) Ann. N.Y. Acad. Sci. 193, 139-144
11. Davidson, B., Neary, J. T., Schwartz, S., Maloof, F., and Soodak, M. (1973) Prep. Biochem. 3, 473-493
12. Neary, J. T., Davidson, B., Armstrong, A., Maloof, F., and Soodak, M. (1973) Prep. Biochem. 3, 486-508
13. Morris, D. R., and Hager, L. P. (1966) J. Biol. Chem. 241, 1763-1768
14. Rombouts, W. A., Schroeder, W. A., and Morrison, M. (1967) Biochemistry 6, 2965-2977
15. Goldstein, I. J., Hollerman, C. E., and Smith, E. E. (1965) Biochemistry 4, 878-883
16. Sharon, N., and Lis, H. (1972) Science 177, 949-959
17. Lis, H., and Sharon, N. (1973) Annu. Rev. Biochem. 42, 541-574
18. Goldstein, I. J. (1975) Adv. Exp Med Biol 55, 35-53
19. Fournier, M., DePreilaune, S., and Nunez, J. (1972) Biochimie 54, 483-492
20. Steinmann, A., and Stryer, I. (1973) Biochim Biophys Acta 129, 1502
21. Allan, D., Auger, J., and Crompton, M. J. (1972) Nature New Biol. 236, 23-25
22. Sica, V., Parikh, I., Nola, E., Puca, G. A., and Cuatrecasas, P. (1973) J. Biol. Chem. 245, 6543-6558
23. Sumner, J. R., and Howell, S. F. (1936) J. Biol. Chem. 115, 583-588
24. Kalb, A. J., and Levitzki, A. (1968) Biochim. Biophys. Acta 109, 669-672
25. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) Biochim. Biophys. Acta 71, 114-123
26. Van Herle, A. J., Uller, R. P., Matthews, N. L., and Brown, J. (1973) J. Clin. Invest. 52, 1330-1337
27. Hosoya, T., Kondo, Y., and U. (1962) J. Biochem (Tokyo) 52, 189-199
28. Hosoya, T. (1962) J. Biochem. (Tokyo) 54, 381-388
29. Lawry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
30. Lang, C. A. (1956) Anal. Chem. 30, 1602-1604
31. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) Anal. Chem. 28, 350-356
32. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1970) Biochemistry 10, 2606-2617
33. Bence, D. J. (1964) Ann. N.Y. Acad. Sci. 121, 404-427
34. Katto, S. M., and Odexman, D. D. (1957) Biochemistry 14, 2292-2298
35. Yariv, J., Kalb, A. J., and Levitzki, A. (1968) Biochim. Biophys. Acta 165, 303-305
36. Takahashi, S., Pollack, J., and Seifter, S. (1974) Biochim. Biophys. Acta 371, 71-79
37. Bae, S., and Rieger, F. (1975) FEBS Lett. 25, 282-286
38. Wiedner, T., Gentinetta, R., and Brodbeck, U. (1974) FEBS Lett. 47, 260-263
39. Dufau, M. L., Tsumurara, T., and Cat, K. J. (1972) Biochim. Biophys. Acta 278, 281-292
40. Ehnholm, G., Shaw, W., Greene, H., and Brown, W. V. (1975) J. Biol. Chem. 290, 6756-6761

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Interaction of Thyroid Peroxidase with Concanavalin A

41. Page, M. (1973) Can. J. Biochem. 51, 1213–1215
42. Jeanloz, R. W. (1966) in Glycoproteins (A. Gottschalk, ed) p. 381, Elsevier, Amsterdam
43. Felgenhauer, K., Weis, A., and Glenner, G. G. (1970) J. Chromatogr. 46, 116–119
44. Adair, W. L., and Kornfeld, S. (1974) J. Biol. Chem. 249, 4696–4704
45. Lenard, J. (1970) Biochemistry 9, 1129–1132
46. Cuatrecasas, P. (1973) Biochemistry 12, 1312–1323
47. Gray, R. D., and Glew, R. H. (1973) J. Biol. Chem. 248, 7547–7551
48. Noonan, K. D., and Burger, M. M. (1973) J. Biol. Chem. 248, 4286–4292
49. Barber, B. H., and Carver, J. P. (1975) Can. J. Biochem. 53, 371–379
50. Becker, J. W., Reeke, G. N., Jr., Wang, J. L., Cunningham, B. A., and Edelman, G. M. (1975) J. Biol. Chem. 250, 1513–1524
51. Hardman, K. D., and Aimsworth, C. F. (1973) Biochemistry 12, 4442–4448
52. Davey, M. W., Huang, J. W., Sulkowski, E., and Carter, W. A. (1974) J. Biol. Chem. 249, 6354–6355
53. Podder, S. K., Surolia, A., and Bachhawat, B. K. (1974) Eur. J. Biochem. 44, 151–160
54. Bislayee, S., and Bachhawat, B. K. (1974) Biochim. Biophys. Acta 334, 378–388
55. Clarke, S. (1975) J. Biol. Chem. 250, 5459–5469
56. Gibbons, R. A. (1972) in Glycoproteins (A. Gottschalk, ed) Part A, pp. 31–140, Elsevier, Amsterdam
Interaction of thyroid peroxidase with concanavalin A covalently coupled to agarose.
J T Neary, D Koepsell, B Davidson, A Armstrong, H V Strout, M Soodak and F Maloof

J. Biol. Chem. 1977, 252:1264-1271.

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