Ferredoxin and Ferredoxin Reductase Activities in Bovine Thyroid
POSSIBLE RELATIONSHIP TO IODOTYROSINE DEIODINASE*

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NADPH-ferredoxin reductase and ferredoxin activities have been identified in bovine thyroid particulate fractions (mainly mitochondria) after ultrasonication and DEAE-cellulose chromatography. The proteins were identified by their ability to reconstitute NADPH-cytochrome c reductase activity when used in combination. NADPH-ferredoxin reductase and ferredoxin also catalyzed NADPH-dependent deiodination of L-diiodotyrosine; bovine adrenodoxin and adrenodoxin reductase could partially replace the thyroidal components in NADPH-dependent deiodination of L-diiodotyrosine. Both these reconstitutive activities were substantially inhibited by the iron chelators, 4,4'-dipyridyl and o-phenanthroline. Deiodination by the NADPH-ferredoxin reductase-ferredoxin system was inhibited by the addition of a previously characterized dithionite-responsive flavoprotein iodotyrosine deiodinase (preparation F), isolated and purified from bovine thyroid particulate fractions after solubilization with steapsin. Ferredoxin reductase alone showed dithionite-responsive deiodinase activity and elution profiles of this activity on gel filtration before and after steapsin treatment suggest that preparation F may be a form of ferredoxin reductase modified by steapsin.

A soluble FMN-containing protein has been purified from thyroid particulate fractions which have been treated with steapsin (1, 2); this flavoprotein catalyzes deiodination of iodotyrosines in the presence of strong reducing agents, such as dithionite, reduced methyl viologen, and reduced clostridial ferredoxin. This flavoenzyme, unlike the particulate preparations from which it is derived, is inactive with NADPH. Moreover, the iron chelator, 4,4'-dipyridyl, considerably inhibited the activity of the particulate preparations without affecting the deiodinating activity of the steapsin-solubilized preparation in the presence of dithionite or reduced methyl viologen. These observations suggested that in the thyroid particulate preparation, electron transport between NADPH and the flavoprotein deiodinase might involve an iron-containing intermediate.

These findings, together with recent demonstrations of ferredoxins and NADPH-ferredoxin reductases in the mitochondria of kidney (3), liver (4), testis (5), and brain (6), prompted us to search for such substances in the thyroid particulate system. In this paper, we describe the solubilization and partial purification of ferredoxin and NADPH-ferredoxin reductase activities from bovine thyroid-particulate fractions and the reconstitution of NADPH-responsive iodotyrosine deiodinase activity in a solubilized system.

EXPERIMENTAL PROCEDURES

Preparation of Bovine Thyroid Particulate Fractions—Cleaned and frozen bovine thyroid glands (500 to 1000 g) were rinsed, minced, and homogenized in a Potter-Elvehjem class-Teflon homogenizer in 4 volumes (w/v) of ice-cold 0.25 M sucrose containing 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4) and 0.5 mM EDTA. The homogenate was centrifuged at 700 × g for 10 min at 4°C. The pellet was resuspended once and the combined supernatants were centrifuged at 100,000 × g for 90 min. The pellet so obtained was resuspended and washed three times in the homogenizing buffer and processed further for the preparation of either thyroid ferredoxin and thyroid ferredoxin reductase or the steapsin-solubilized deiodinase (preparation F) as detailed below.

Isolation and Partial Purification of NADPH-ferredoxin Reductase and Ferredoxin from Bovine Thyroid Particulate Fractions—The method used was essentially that described by Foster and Wilson (7) for isolation of adrenal ferredoxin and NADPH-ferredoxin reductase with some modifications of the DEAE-cellulose chromatography step and is summarized in Fig. 1. In the final chromatography step (Fig. 1), the precipitate obtained between 35 and 65% saturation of the sonicated extract with (NH₄)₂SO₄ was dialyzed against 10 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 1 mM mercaptoethanol and applied to a DEAE-cellulose column (1.5 × 30 cm) equilibrated with the same buffer. The column was then washed successively with 150 ml of the eluting buffer and with 150 ml of the same buffer containing 0.1 M KCl. At the end of the second elution, a precipitate obtained by raising the 35 to 60% (NH₄)₂SO₄ supernate to 85% saturation with respect to (NH₄)₂SO₄ (dissolved in and dialyzed against the equilibrating buffer containing 0.1 M KCl) was applied to the column. The column was then eluted with a linear gradient generated from 20 ml of the eluting buffer containing 0.1 M KCl and 250 ml of the same buffer containing 0.5 M KCl. The elution was completed with 50 ml of the equilibrating buffer containing 0.5 M KCl. One-milliliter fractions were collected during the stepwise elutions and 5-ml fractions were collected during the gradient elution. The NADPH-ferredoxin reductase activity and ferredoxin-like activities in each fraction were monitored using purified bovine adrenodoxin (1.5 nmol) and adrenodoxin reductase (0.5 units), respectively. The fractions were also monitored for NADPH-diaphorase and ferredoxin-dependent NADPH-cytochrome c reductase activities. The results are expressed as enzymatic activity units/ml of fractions.

Other Enzymes—Iodotyrosine deiodinase (preparation F) was prepared from bovine thyroid particulate fractions by methods described earlier (2). Adrenodoxin and adrenodoxin reductase were isolated and purified from bovine adrenal mitochondria by the method of Foster and Wilson (7).

Enzyme Assays—NADPH cytochrome c reductase activities were assayed spectrophotometrically and the rate of reduction of cytochrome c (ΔA at 550 nm) was recorded at room temperature on a Gilford model 2000 recording spectrophotometer in the following manner. The sample and the reference cuvettes contained, in 800 μl of 0.1 M Mops' buffer (pH 7.4), 75 nmol of cytochrome c in 1 μmol of

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The abbreviation used is: Mops, 4-morpholinepropanesulfonic acid.
**RESULTS**

Isolation of Ferredoxin Reductase and Ferredoxin from Bovine Thyroid Particulate Fractions—The elution profile from the DEAE-cellulose column is shown in Fig. 2. As shown in the figure, two enzyme activities relative to flavoproteins (as judged by an A_415nm/A_346nm ratio) were eluted in the nonretained fractions, one being a NADPH-dichloroindophenol-diaphorase activity emerging in the earlier fractions, and the other an adrenodoxin-dependent NADPH-cytochrome c reductase (eluted in later fractions). In contrast to the earlier peak (Peak I), the activity in the later peak (Peak IIa) was dependent on the presence of adrenodoxin and is designated as the thyroid ferredoxin reductase. The bulk of the thyroid ferredoxin reductase activity, however, emerged on stepwise elution at 100 mM KCl (Peak IIb). Since Peak IIa activities were somewhat contaminated with NADPH-diaphorase activities, the fractions under the two peaks (IIa and IIb) were pooled separately and concentrated by precipitation at 80% saturation with ammonium sulfate and dialysis against 10 mM Mops buffer (pH 7.4). The preparation (Peak IIb) had an activity of 1.93 nmol of cytochrome c reduced/mg of protein/min in the presence of 0.3 μm bovine adrenodoxin. The enzyme was highly unstable and the total activity obtained varied from experiment to experiment but once prepared, the enzyme could be stored at -70°C for several weeks. The aged enzyme, however, required FAD in the incubation medium for optimal activity. Two peaks (Peaks III and IV), showing NADPH cytochrome c reductase activities, emerged during elution with a salt gradient and stepwise elution with 0.5 M KCl. Of these, the activity in Peak III, eluting at about 0.3 M KCl and corresponding to a flavoprotein peak, could be obtained by the addition of NADPH alone. This activity, presumably, represents the NADPH-cytochrome c reductase of the microsomes and outer mitochondrial membranes (3). The NADPH-cytochrome c reductase activity of fractions included under Peak IV, however, was dependent on the addition of adrenodoxin reductase to the assay medium. This activity also corresponded to a small peak of A_415nm/A_346nm ratio with little absorbance at 450 nm. Aliquots of Peak II fractions (thyroid ferredoxin reductase) were an effective substitute for adrenodoxin reductase for the NADPH-dependent cytochrome c reductase of Peak IV material. Peak IV then seems to contain thyroid ferredoxin. In some preparations, Peak IV fractions were contaminated with ferredoxin-independent NADPH-cytochrome c reductase activities due to overlapping of the Peak III and IV activity peaks. The two activities were separated by a second DEAE-cellulose chromatography. The fractions under Peak IV were pooled, concentrated on Amicon UM-2 membrane filters, and dialyzed against 10 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM KCl. This was then applied on a DEAE-cellulose column (1.5 × 15 cm) equilibrated with the same buffer. A linear gradient of KCl (100 to 300 mM) in 10 mM potassium phosphate buffer (pH 7.4) in a total volume of 500 ml was then applied to the column. Most of the adrenodoxin reductase-independent NADPH-cytochrome c reductase activity was eluted out by the gradient. A reddish yellow band that was formed on top of the column was then carefully removed, suspended in a small volume of 10 mM KPi buffer containing 0.5 mM KCl and re-packed in a small pencil-size glass column. The column was then eluted with 100 ml of 10 mM KPi buffer containing 0.5 mM KCl and the eluate was concentrated on an Amicon UM-2 membrane. This procedure resulted in about 2-fold purification of ferredoxin (153 units/mg of protein) compared to the first DEAE-cellulose preparation. This preparation was characterized by an absorption maximum at 410 nm with a slight shoulder around 455 nm and some absorbance.
Thyroid Ferredoxin and Ferredoxin Reductase

Figure 2. Elution profile obtained by DEAE-cellulose chromatography of a sonicated extract of bovine thyroid particulate fractions precipitated with ammonium sulfate. A precipitate, obtained between 35 and 65% saturation with ammonium sulfate, of the sonicated extract was first applied to the DEAE-cellulose column equilibrated with 10 mM KPi, 1 mM EDTA, and 1 mM mercaptoethanol (pH 7.4) and elution was started as indicated; a precipitate obtained by further raising the ammonium sulfate concentration to 85% was subsequently applied to the column before starting the 0.1 to 0.5 M KCl gradient, as shown in Fig. 1. Enzymatic assays for the activities of NADPH-diaphorase, ferredoxin, and ferredoxin-dependent or independent NADPH-cytochrome c reductases were performed in each fraction by methods as detailed in the text. Each point represents activity in units/ml. The same scale (left ordinate) was used for plotting the ferredoxin-dependent or independent NADPH-cytochrome c reductase activities. 

Subcellular Distribution of Ferredoxin and Ferredoxin Reductase—For maximum yields, a pellet obtained by high speed centrifugation (100,000 × g) of the postnuclear supernatant was used for the preparative procedures. When processed separately, the bulk of the activities were contained in the mitochondrial fraction, (pellet obtained by centrifuging postnuclear supernatant at 20,000 × g for 20 min) although a small amount of activity could also be detected in the microsomal fractions (pellet obtained by centrifuging postmitochondrial fraction at 100,000 × g for 60 min). Whether this was due to contamination with mitochondrial fractions remains to be determined. Moreover, owing to the much higher content of NADPH-cytochrome c reductase activity in microsomal preparations, purer preparations of ferredoxin could be obtained from mitochondria than from microsomes. For these reasons, mitochondrial preparations alone were used for experiments aimed at isolation of ferredoxin only. A rapid method employing a reversed (NH₄)₂SO₄-DEAE-cellulose chromatography procedure, as described by Mayhew (13), gave preparations of ferredoxin comparable, in yields and activities, to those obtained by methods as described above. For most procedures, aimed at obtaining both ferredoxin and its reductase from the same thyroid homogenate preparations, the particulate fraction, containing both mitochondria and microsomes, was used as the starting material.

Reconstitution of NADPH-cytochrome c Reductase Activity—Reconstitution was performed by using thyroid ferredoxin reductase and thyroid ferredoxin as obtained above. In the presence of excess thyroid ferredoxin reductase, the amount of cytochrome c reduced/min was proportional to the amount of thyroid ferredoxin added (Fig. 4). Conversely, in the presence of excess thyroid ferredoxin, the rate of cytochrome c reduction increased with increasing amounts of around 550 nm (Fig. 3). The 410 nm peak and the shoulder at 455 nm were unaffected by cyanide but disappeared upon reduction with dithionite concomitant with the appearance of a new maximum around 420 nm. These features are very similar to those reported for liver (4, 12) and brain (6) ferredoxins. The preparation still contained small amounts of NADPH-cytochrome c reductase activity that was independent of the presence of adrenodoxin reductase. Attempts to obtain a ferredoxin preparation completely free from adrenodoxin reductase-independent NADPH-cytochrome c reductase activity have not so far been successful.

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thyroid ferredoxin reductase (Fig. 5). The ferredoxin-dependent NADPH-cytochrome c reductase activity was completely inhibited by \( \alpha, \alpha' \)-dipyridyl (1 mM). The rate of cytochrome c reduction was not affected by the addition of superoxide dismutase (75 units/ml) at any concentration of thyroid ferredoxin reductase or thyroid ferredoxin. The activities of thyroid ferredoxin reductase with various ferredoxins are shown in Table I. Thyroid ferredoxin reductase was most active with thyroid ferredoxin, showing about 30% less activity with bovine adrenodoxin and was only minimally active with clostridial ferredoxin (Table I). The relatively specific requirement for thyroidal ferredoxin for thyroid ferredoxin reductase activity becomes apparent when the activities of the relatively crude thyroid ferredoxin preparations are compared with those of much more purified preparations of adrenodoxin and clostridial ferredoxins used in these experiments. The adrenodoxin used had a specific activity of about 800 units/mg of protein in the presence of 25 pmol of purified adrenal NADPH-adrenodoxin reductase; the corresponding value for clostridial ferredoxin was approximately 1200 in the presence of excess spinach NADPH-ferredoxin reductase; the specific activity of thyroid ferredoxin obtained with 25 pmol of adrenodoxin reductase was 120 units/mg of protein. The data presented in Table I thus show a 10-fold increase in specific activity of a relatively crude preparation of thyroid ferredoxin when assayed with thyroid ferredoxin reductase, as compared with adrenodoxin reductase, suggesting that the thyroidal and adrenal ferredoxin systems are not identical.

**Reconstitution of NADPH-responsive Deiodinase Activity**—While thyroid ferredoxin reductase or thyroid ferredoxin alone had little deiodinating activity in the presence of NADPH, reconstitution of NADPH-responsive deiodinase activity, comparable to that obtained with dithionite, could be achieved when thyroid ferredoxin reductase was incubated in the presence of thyroid ferredoxin (Fig. 6). This activity was almost completely inhibited in the presence of 1 mM dipyridyl, indicating an essential role of ferredoxin in NADPH-mediated deiodination. The activity was concentration-dependent with respect to both ferredoxin reductase (Fig. 7) and ferredoxin (Fig. 8).

**Dithionite-responsive Deiodinase Activity**—Thyroid ferredoxin reductase, as prepared above, showed dithionite-responsive iodothyroside deiodinase activity (Fig. 6) in the absence of added ferredoxins. This activity was not affected by dipyridyl (1 mM). The specific deiodinase activity with dithionite was about 10% of similar preparations obtained with \((NH_4)_2SO_4\) precipitation and DEAE-cellulose chromatography after steapsin digestion. This may be related partly to a quantitatively greater solubilization of bulk proteins from the particulate fractions by the sonication procedure (+48%, see Table II) as compared to steapsin digestion (20 to 25%, see Ref. 2) and partly to a much less effective extraction of dithionite-responsive deiodinase activity with sonication as compared to that obtained with steapsin digestion. As shown in Table II, sonication of the particulate fraction released only 20% of the deiodinating activity into the supernatant, with considerable activity remaining in the pellet, as opposed to steapsin digestion which released virtually all the activity into the supernatant (2). The sonicated supernatant had, in effect, a decreased specific deiodinating activity compared to microsomes (Table II), suggesting co-extraction of a greater proportion of inert proteins. Further purification of both preparations gave parallel increases in specific activities with about 11-fold purification after DEAE-cellulose chromatography.

**Effects of Preparation F on the Deiodinating Activity of the Thyroid Ferredoxin Reductase-Thyroid Ferredoxin System**—Addition of a steapsin-solubilized and purified deiodinase preparation F inhibited substantially the NADPH-dependent deiodinating activity of the ferredoxin system without affecting the dithionite-responsive deiodinase activity of thyroid ferredoxin reductase (Fig. 6). The dithionite-responsive deiodinating activity of a thyroid ferredoxin preparation was not inhibited by Preparation F, with similar inhibition occurring in the presence or absence of steapsin (Fig. 6).

**Table I**

| Electron carrier | NADPH-cytochrome c reductase activity (milliunits/mg thyroid ferredoxin reductase protein) |
|------------------|---------------------------------------------------------------------------------------------|
| None             | 15 ± 2 (8)                                                                                  |
| Bovine adrenodoxin| 5790 ± 175 (8)                                                                              |
| Bovine thyroid ferredoxin| 8450 ± 250 (8)                                                                 |
| Clostridial ferredoxin| 210 ± 35 (4)                                                                 |

The assay medium contained, in a total volume of 1 ml: 300 \( \mu \)mol of Mops (pH 7.4), 100 \( \mu \)mol of NADPH, 30 \( \mu \)mol of cytochrome c, thyroid ferredoxin reductase (TFdR) in varying amounts, and thyroid ferredoxin (TFd) with amounts fixed at either 50 or 100 \( \mu \)g. The rate of cytochrome c reduction was monitored by the rate of increase in absorbance at 550 nm. Each point represents the mean of closely agreeing triplicate determinations.
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| Iodotyrosine Deiodinase Activity (Units) | Additions (µg/ml) |
|-----------------------------------------|------------------|
|                                         | TfdR (370)       |
|                                         | Tfd (110)        |
|                                         | Ad (130)         |
|                                         | F (1.1)          |
|                                         | Dipryndyl (156)  |
| 0                                       | -                |
| 2                                       | -                |
| 4                                       | -                |
| 6                                       | -                |
| 8                                       | -                |
| 10                                      | -                |
| 1                                       | TfdR             |
| 2                                       | Tfd              |
| 3                                       | Ad               |
| 4                                       | F                |
| 5                                       | Dipryndyl        |

FIG. 6. Reconstitution of NADPH-responsive deiodinase activity with the ferredoxin-ferredoxin reductase system. The assay of deiodinase activity was based on the liberation of $^{125}$I from $[^{125}]$-iodotyrosine by methods as described in the text. The amounts of various components used are shown in parentheses. TfdR, thyroid ferredoxin reductase; Tfd, thyroid ferredoxin; Ad, bovine adrenal ferredoxin; F, steapsin-solubilized and purified iodotyrosine deiodinase. Each estimation was done in duplicates which were in excellent agreement. Comparable results were obtained in repeat experiments using different preparations of thyroid ferredoxin reductase, thyroid ferredoxin, and F.

dehiodinase activities of mixtures of thyroid ferredoxin reductase with preparation F were, in fact, additive. The possibility that the inhibitory effect of preparation F on the NADPH-responsive deiodinase activities of the thyroid ferredoxin reductase-thyroid ferredoxin system was due to competition of preparation F with thyroid ferredoxin reductase-thyroid ferredoxin for the substrate L-diiodotyrosine was then considered, since preparation F, although not catalytically active with NADPH, was active in substrate binding, as judged by dithionite-responsive deiodination of L-diiodotyrosine. The results presented in Fig. 9 show that the inhibitory effect of preparation F on NADPH-responsive deiodinase activity could be overcome with increasing concentrations of L-diiodotyrosine. The apparent $K_m$ of the thyroid ferredoxin reductase-thyroid ferredoxin system for L-diiodotyrosine was 1.2 μM, as compared with a $K_m$ of 1.02 μM for the steapsin solubilized preparation F at a similar stage of purification (ammonium sulfate precipitation and DEAE-cellulose chromatography (1)); this was raised to 9.6 μM in the presence of 1.1 µg of purified preparation F. Preparation F was calculated to have a $K_m$ of 20 nM in this reaction.

The dithionite-responsive deiodinase activity of thyroid ferredoxin reductase and the inhibitory effects of preparation F on the NADPH-responsive deiodinase activity of the thyroid ferredoxin reductase-thyroid ferredoxin system also raise the possibility that preparation F may represent a form of thyroid ferredoxin reductase modified by the lipase and proteolytic activities of steapsin in such a way that it cannot utilize NADPH or ferredoxin for deiodinating activity. To test this, a thyroid ferredoxin reductase preparation (900 µg) was incubated overnight with 0.075% steapsin and was then subjected to gel filtration on Sephadex G-100. The fractions were then tested for dithionite-responsive deiodinase activities. Comparison of the elution pattern so obtained with those obtained with untreated thyroid ferredoxin reductase and preparation F is shown in Fig. 10. Gel filtration of untreated thyroid ferredoxin reductase shows a major deiodinating component with an apparent molecular weight of 67,700 as cal-

FIG. 7. Dependence of NADPH-responsive iodotyrosine deiodinase activity on the amount of ferredoxin reductase added in the presence of a fixed amount of ferredoxin (100 µg). Assay of deiodinase activity was performed as described in the text. The points represent means of closely agreeing triplicates. TfdR, thyroid ferredoxin reductase.

FIG. 8. Ferredoxin-dependent iodotyrosine deiodinase activity of ferredoxin reductase: effects of preparation F. The assay of deiodinase activity and the incubation conditions are as described in the text. Estimations, in triplicate, were in excellent agreement.
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The mitochondrial-microsomal pellet was divided into two equal parts and then subjected to sonication or steapsin-digestion, as described in the text. The steapsin-solubilized preparation was inactive with NADPH in the reduction of cytochrome c in the presence of ferredoxin. The pellet and the sonicated preparation showed 42,000 (2), the characteristics of which corresponded to that of preparation F, previously characterized as having a molecular weight of 

\[ \text{Calculation by using equations as proposed by Determan and Michel (14).} \]

| Preparation         | Total protein | NADPH-ferredoxin reductase activity | Dithionite-responsive iodotyrosine deiodinase activity | Purification |
|---------------------|---------------|------------------------------------|------------------------------------------------------|--------------|
|                     | mg            | units \( \times \text{mg}^{-1} \) | units \( \times \text{mg}^{-1} \) | -fold |
| 1. Mitochondrial-microsomal pellet | 7335 | 34,800 | 4.7 |
| 2. Supernatant       |               |                                   |                                                      |              |
| a) Steapsin-treated  | 815           | 16,625                             | 20.4                                                 | 1            |
| b) Sonicated         | 1756          | 3,150                              | 1.8                                                  | 1            |
| 3. \((\text{NH}_4)\text{SO}_4\) precipitate | 284 | 10,900 | 38.4 | 1.9 |
| a) Steapsin-treated  | 880           | 3,960                              | 4.5                                                  | 2.5          |
| b) Sonicated         |               | 4,480                              | 224                                                   | 11.0         |
| 4. DEAE-cellulose eluate | 20       | 4,480                              | 224                                                   | 11.0         |

**DISCUSSION**

The data presented in this paper indicate the presence of a ferredoxin-like substance in thyroid particulate fractions. The criteria used for identifying these substances have been: 1) the ability to reduce cytochrome c in the presence of NADPH only when used in combination, 2) elution behavior from DEAE-cellulose closely resembling those of ferredoxin and ferredoxin reductase iso-

**Fig. 9. Lineweaver-Burk plot of the NADPH-mediated deiodinase activity of the thyroid ferredoxin system in the presence or absence of preparation F.** Incubation conditions and assay of deiodinase activity are described in the text. The data are representative of three similar experiments. **TFd, thyroid ferredoxin; TFdR, thyroid ferredoxin reductase; L-DIT, L-diodotyrosine.**

**Fig. 10. Effect of steapsin treatment on elution behavior of thyroid ferredoxin reductase from Sephadex G-100.** Thyroid ferredoxin reductase (TFdR) (900 pg) was treated overnight with 0.07% steapsin in 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1 ml and was then subjected to gel filtration in the same buffer on Sephadex G-100 (column size, 1 × 65 ml). Untreated thyroid ferredoxin reductase (900 pg) and preparation F (F) (1.5 pg) were then chromatographed separately on the same column under identical conditions. Aliquots of fractions were then assayed for dithionite-responsive deiodinase activity. The ordinate shows the percentage of L-diodotyrosine deiodinated using the usual assay conditions. The data are representative of four similar experiments.

**TABLE II**

Solubilization of NADPH-ferredoxin reductase and dithionite-responsive deiodinase activities from bovine thyroid particulate fractions: comparison between sonication and steapsin treatment.

| Preparation                        | Total protein | NADPH-ferredoxin reductase activity | Dithionite-responsive iodotyrosine deiodinase activity | Purification |
|-------------------------------------|---------------|------------------------------------|------------------------------------------------------|--------------|
|                                     | mg            | units \( \times \text{mg}^{-1} \) | units \( \times \text{mg}^{-1} \) | -fold |
| 1. Mitochondrial-microsomal pellet  | 7335          | 34,800                             | 4.7                                                  |              |
| 2. Supernatant                      |               |                                    |                                                      |              |
| a) Steapsin-treated                 | 815           | 16,625                             | 20.4                                                 | 1            |
| b) Sonicated                        | 1756          | 3,150                              | 1.8                                                  | 1            |
| 3. \((\text{NH}_4)\text{SO}_4\) precipitate | 284 | 10,900 | 38.4 | 1.9 |
| a) Steapsin-treated                 | 880           | 3,960                              | 4.5                                                  | 2.5          |
| b) Sonicated                        |               | 4,480                              | 224                                                   | 11.0         |
| 4. DEAE-cellulose eluate            | 20            | 4,480                              | 224                                                   | 11.0         |
lated from: other mammalian tissues, and 3) appearance of ferredoxin-like activities in fractions displaying \( A_{415 \text{ nm}}/A_{280 \text{ nm}} \) ratios above the base-line along with a \( \lambda_{\text{max}} \) at 410 nm of the preparation obtained by pooling these fractions. Although the soluble ferredoxin isolated from different mammalian tissues have so far been shown to function mainly in cytochrome P-450 dependent hydroxylation reactions, their possible participation in other low potential oxidation-reduction reactions of animal cells cannot be excluded, and an impressive array of participation in other low potential oxidation-reduction reactions of

particular interest is the ability of purified bovine adrenodoxin-adrenodoxin reductase to deiodinate diiodotyrosine in the presence of NADPH, albeit at a much lower rate than the thyroidal system (Fig. 5). This raises the possibility of other physiological roles of ferredoxins in addition to steroid and drug hydroxylations. In this context, it is interesting to note that a cytochrome P-450-dependent steroid hydroxylation activity could not be detected in ferredoxin preparations isolated from rat brain (6).

The flavoenzyme F has a highly negative oxidation-reduction potential (−0.412 V at pH 7, 25°C) for the couple semiquinone/fully reduced enzyme (16) and requires, for its deiodinating activity, strong reductants of highly negative midpoint potentials such as dithionite or reduced methyl viologen, which fully reduce the enzyme (16). It was also observed that preparation F, which was itself inactive with NADPH, could be restored to full deiodinating activity by the addition of clostridial ferredoxin (\( E^0 = −0.420 \text{ V} \)) and spinach ferredoxin reductase in the presence of NADPH (17). This activation, like that with dithionite and reduced methyl viologen, was apparently achieved through full reduction of the flavin moiety of the enzyme F since 1) this ferredoxin system did not display any intrinsic deiodinase activities, either with NADPH or dithionite, in the absence of preparation F and 2) spectral studies indicated full reduction of added flavins by this ferredoxin system in the presence of NADPH (17). This raised the possibility that the thyroid may contain a similar ferredoxin system that may play a role in intrathyroidal deiodination of iodothyronines.

The data presented in this communication do indicate the presence of ferredoxin and NADPH-ferredoxin reductase in the thyroid gland. However, it soon became apparent that the thyroidal ferredoxin system (thyroid ferredoxin reductase-thyroid ferredoxin) was fundamentally different in its characteristics from the spinach-clostridial system. First, not only did the thyroid ferredoxin reductase-thyroid ferredoxin system display NADPH-responsive deiodinating activities in the absence of preparation F, but thyroid ferredoxin reductase alone was active with dithionite. Second, in sharp contrast to the spinach-clostridial ferredoxin system which activated preparation F in the presence of NADPH, addition of preparation F to the thyroid ferredoxin reductase-thyroid ferredoxin system substantially inhibited its NADPH-responsive deiodinating activities; the dithionite-responsive deiodinating activities were not affected. In fact, they were additive (Fig. 6). This inhibition could, however, be overcome at higher substrate concentrations (Fig. 9) suggesting that the two deiodinating systems under study, thyroid ferredoxin reductase and thyroid ferredoxin on the one hand and preparation F on the other, were competing for the iodothyronine substrate. This substrate competition, when considered along with (a) the additive nature of dithionite-responsiveness and (b) the conversion of thyroid ferredoxin reductase by steapsin treatment to a form resembling preparation F in its gel filtration pattern (Fig. 10), would raise the possibility that the two extracted deiodinating systems may originate from a single deiodinating mechanism in situ. In this model, preparation F may be conceived as being generated by the proteolytic activities contaminating steapsin preparations from the native thyroid ferredoxin reductase; the product F has lost the ability to interact with thyroid ferredoxin in transferring the reducing equivalents of NADPH to the enzyme flavin for its full reduction that is needed for activity. Dithiothreitol or other sufficiently strong reductants can bypass the need for interaction with thyroid ferredoxin and directly reduce the enzyme flavin by a 2 e⁻ transfer (a prerequisite for its activation). Such a model is analogous to those reported for NADPH-cytochrome b₅ reductase (18) and NADPH-cytochrome P-450 reductase (19) where the failure of the trypsin- or steapsin-solubilized enzymes to interact with the natural substrates has been ascribed to the cleavage of a hydrophobic terminal sequence. As we have reported earlier (2), NADPH can only half-reduce the flavin of F; for the full reduction of the native enzyme in situ, a ternary complex involving the enzyme (thyroid ferredoxin reductase), thyroid ferredoxin, and NADPH, as has been suggested for the adrenodoxin reductase-adrenodoxin system (20), may be needed. The formulation of an appropriate model depicting the interrelationships between these two deiodinating systems and the physical nature of the actual electron transfer mechanism must, however, await detailed studies of the characteristics of more purified preparations, especially including stoichiometric studies of preparation F generated from much more purified thyroid ferredoxin reductase, as well as further studies of the competitive nature of the inhibition between thyroid ferredoxin reductase and preparation F.

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