Isolation and Characterization of Calf Liver Thioredoxin*

NIELS-ERIK ENGSTRÖM, ARNE HOLMGREN,† AGNE LARSSON,§ AND STEFAN SÖDERHÄLL

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SUMMARY

The reduced form of thioredoxin, a low molecular weight hydrogen transport protein, was isolated from calf liver. The purification involved heat treatment and chromatography on DEAE-cellulose, Sephadex G-50, and CM-cellulose, and it resulted in an apparently pure protein after a 4,000-fold purification with a final yield of 30%. The thioredoxin preparation obtained was homogeneous as judged by polyacrylamide gel electrophoresis and the presence of valine as the only NH₂-terminal amino acid. Chromatography on Sephadex indicated a molecular weight of approximately 12,000 for liver thioredoxin and amino acid analysis showed a content of 103 amino acid residues, including 4 half-cystine residues. Oxidation in vitro of the reduced form of calf liver thioredoxin indicated that only 2 of the half-cystine residues formed an oxidation-reduction-active disulfide bridge.

Thioredoxin (1) is a low molecular weight hydrogen transport protein which was originally isolated from Escherichia coli. The oxidized form (thioredoxin-S₂) contains an oxidation-reduction-active disulfide bridge. This disulfide is reduced to 2 half-cystine residues by NADPH in a reaction catalyzed by a specific enzyme, thioredoxin reductase (2) (Reaction I). The reduced form of thioredoxin (thioredoxin-(SH)₂) is reoxidized in the enzymatic reduction of ribonucleotides (3) (Reaction II) or in non-enzymatic reduction of a disulfide-containing compound (X-S₂) (Reaction III) as shown below:

\[
\text{Thioredoxin-}-(SH)_2 + X-S_2 \rightarrow \text{thioredoxin-S}_2 + X-(SH)_2 \quad \text{(III)}
\]

Both thioredoxin and thioredoxin reductase from E. coli have been studied extensively (4, 5). Thioredoxin has also been purified from Lactobacillus leichmanni (6), yeast (7), and phage T4-infected E. coli (8).

Evidence of a mammalian thioredoxin was first obtained by Moore using rat Novikoff hepatoma (9, 10). Recently a thioredoxin was also identified and obtained as an approximately 50% pure preparation from rat liver (11).

The objective of the present investigation was to isolate and characterize a homogeneous liver thioredoxin and calf liver turned out to be a suitable starting material for large scale preparations.

EXPERIMENTAL PROCEDURE

Materials

Livers from young calves (age 7 to 14 days) were purchased from Farmek (Sweden). The livers were frozen immediately after removal from the animals and were stored without any further treatment at -20° until used. Ribonucleotide reductase from E. coli purified through the first DEAE-cellulose chromatography step (12) was a preparation available in this laboratory. Thioredoxin reductase from calf liver was purified approximately 500-fold by chromatography on Sephadex G-50, DEAE-cellulose, TEAE-cellulose (triethylaminoethylcellulose), and Sephadex G-200.† Sephadex G-25 and G-50 were from Pharmacia AB (Sweden). DEAE-cellulose and TEAE-cellulose were obtained from Serva Entwicklungslabor (West Germany). CM-cellulose was a product of Whatman (England). CM-cellulose was a product of Whatman (England). Thioredoxin-(SH)_2 + X-S_2 \rightarrow \text{thioredoxin-S}_2 + X-(SH)_2 (III)

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Methods

Protein Determination

During the early steps in the purification of liver thioredoxin, protein was determined by the method of Lowry et al. (13). After the DEAE-cellulose chromatography the protein concentrations assayed by the Lowry method agreed well with those obtained by analysis of ultraviolet absorption. Therefore protein was determined as the difference between the absorbance obtained by analysis of ultraviolet absorption. Therefore protein was determined by the method of Lowry et al. (13).

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Protein Determination

Ultraprotein

Dilute pools of thioredoxin activity were often obtained during the purification. Such protein was determined by means of ultraviolet absorption at 4\(^{\circ}\) in Diaoio cells (models 52 or 402) purchased from Amicon (Holland) with the use of Diaoio PM-10 membranes. Compressed N\(_2\) was used as the pressure source.

Analytical polyacrylamide gel electrophoresis

Analytical polyacrylamide gel electrophoresis was carried out with a Shandon SAE 2734 disc gel electrophoresis apparatus by a minor modification of the method of Ornstein (14) and Davies (15) and the pH 8.4 buffer system was used. Calf liver thioredoxin (15 to 40 mg) was preincubated for 30 min with 2 mM dithiothreitol in 20 \(\mu\)l of the application buffer. The electrophoresis was carried out in 7.5% polyacrylamide gels at 15 volts per cm for 90 min. The gels were stained with 0.25% Coomassie brilliant blue in water, overnight, and destained with ethanol-acetic acid-water (3:8:1, by volume).

Determination of Extinction Coefficient of Thioredoxin

The extinction coefficient at 280 nm (\(E_{280}\)) for calf liver thioredoxin was calculated on the basis of dry weight determinations. Therefore, thioredoxin was desalted on a column of Sephadex G-25, which had been thoroughly washed with water adjusted to pH 6.5 with ammonia. The absorbance of the desalted protein was determined by dilution of an aliquot in 50 mM Tris-Cl buffer, pH 7.6. The remainder was divided in portions and dried to constant weight as described by Brown et al. (12).

Analytical gel chromatography

The molecular weight of thioredoxin was estimated by gel chromatography on a column of Sephadex G-50 (1.2 x 160 cm) which had been equilibrated with 10 mM potassium phosphate, pH 7.0, 1 mM EDTA, 1 mM dithiothreitol. Bovine serum albumin, thioredoxin from E. coli and NaCl were used as molecular weight markers to calibrate the column.

Amino Acid Analysis

Salt-free, lyophilized samples of calf liver thioredoxin (0.20 mg) were hydrolyzed in duplicate with 1.0 ml of 6 M HCl for 24 and 72 hours at 110\(^{\circ}\) in carefully evacuated tubes. The amino acid compositions of the hydrolysates were determined according to the method of Spackman et al. (16) with a Spinco 120 B amino acid analyzer equipped with high sensitivity cuvettes (17). Half-cystine was determined as cysteic acid after performic acid oxidation at 0\(^{\circ}\) by the method of Hirs (18). Tryptophan was estimated spectrophotometrically by the method of Benece and Schmid (19).

NH\(_2\)-terminal Analyses

The NH\(_2\)-terminal amino acid of thioredoxin was determined by the dansyl method of Gray and Hartley (20), as previously described by Holmgren and Rechard (21). Dansyl-amino acids were identified by two-dimensional thin layer chromatography on polyamide sheets in four solvent systems (22, 23).

Carboxymethylation of Thioredoxin

Carboxymethylation was performed according to the method of Crestfield et al. (24) with minor modification as described by Holmgren (25) for thioredoxin from E. coli. The carboxymethylation was made with [\(^{14}\)C]iodoacetic acid (specific activity 2.8 x 10\(^{4}\) cpm per \(\mu\) mole).

Peptide Map

A peptide map of a tryptic digest of carboxymethylated liver thioredoxin (0.60 mg) was made by paper electrophoresis and chromatography as described by Holmgren (25). \([\(^{14}\)C]Carboxymethylcysteine-containing peptides were located by autoradiography. The exposure of the film (Ilford industrial x-ray film) was made for 60 hours in the dark prior to staining of the paper with ninhydrin.

Determination of Sulphydryl Groups

Free sulphydryl groups of thioredoxin were determined with the DTNB method as described by Ellman (26). The reduced form of thioredoxin was obtained by treatment with dithiothreitol (2 mM) and the remainder of this reducing agent was removed on a column of Sephadex G-25 with deaerated, nitrogen-equilibrated 50 mM, Tris-Cl, 1 mM EDTA, pH 8.0.

Thioredoxin Assays

Three different methods were used to determine the enzymatic activity of thioredoxin.

Method 1—The method was based on the fact that the reduced form of calf liver thioredoxin functions as hydrogen donor for ribonucleotide reductase from E. coli. This assay was only used in early purification steps to test for a good source of starting material and to ensure that a thioredoxin was purified. The conditions for this assay were those of Larson and Larsson (11).

Method 2—This assay was based on the fact that the reduced form of thioredoxin is able to reduce disulfide-containing peptides and proteins (Reaction III). We found that insulin was reduced by calf liver thioredoxin when the reaction was coupled to NADPH and thioredoxin reductase. It was thus possible to get a system where "catalytic" amounts of thioredoxin could be analyzed using NADPH as the hydrogen donor for reduction of insulin disulfides. Free sulphydryl groups formed in insulin were measured spectrophotometrically after stopping the enzymatic reaction with 6 M guanidine hydrochloride containing DTNB.

The incubation mixture (0.12 ml) contained: 0.25 mg of insulin, 0.6 \(\mu\)mole of EDTA, 10 \(\mu\)mole of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, pH 7.6, 0.11 \(\mu\)mole of NADPH, and 10 \(\mu\)g of thioredoxin reductase plus an unknown amount of thioredoxin. After incubation for 15 min at 37\(^{\circ}\) the reaction was stopped by the addition of 0.50 ml of 6 M guanidine hydrochloride, 50 mM Tris-Cl, pH 8.0, containing 10 mM DTNB. The absorbance at 412 nm was measured after 5 min against an incubation mixture without thioredoxin. One unit of thioredoxin activity was defined arbitrarily as the amount which promoted the formation of 1.0 \(\mu\)mole of sulphydryl groups under the above conditions.
As shown in Fig. 1, the assay could be used at all stages of purification. It was rapid and sensitive and especially well suited for locating thioredoxin during column chromatography steps.

**Method 3—**At pH 7 the oxidized form of thioredoxin can be reduced by NADPH and thioredoxin reductase (Reaction 1). It is thus possible to determine the amount of oxidized thioredoxin in a sample by measuring the amount of NADPH which disappears during the reaction.

Each of two cuvettes contained: 50 nmoles of NADPH, 50 μmoles of potassium phosphate buffer, pH 7.0, and 5 μmoles of EDTA in a final volume of 0.50 ml. Thioredoxin was added to one of the cuvettes which was used as a blank in the spectrophotometer. After a zero time reading at 340 nm, 6 μg of thioredoxin reductase were added to each cuvette and the increase in absorbance at 340 nm was recorded at different time intervals. A constant reading should be obtained after 5 min. From the differences between the zero time reading and the final value, the amount of NADPH oxidized was calculated by use of the known molar extinction coefficient (6.2 × 10⁵) for NADPH.

**RESULTS**

In a previous study of liver thioredoxin (11) male Sprague-Dawley rats were used. However, for large scale purification of thioredoxin a more abundant starting material was required. Determination of thioredoxin activity by measuring dCDP formation in the presence of ribonucleotide reductase from E. coli (Method 1) showed that liver from young calves and the male Sprague-Dawley rat contained approximately equal concentrations of thioredoxin. The preparations were therefore started from young calf livers.

Unless otherwise stated, all manipulations were carried out at 4°. Centrifugations were performed at 16,000 × g in a Sorvall model RC2 centrifuge. All buffers contained 1 mM EDTA and, unless otherwise stated, buffers in column chromatographic steps contained 0.1 mM dithiothreitol. Incubations with dithiothreitol prior to chromatographic steps were done for 30 min with a final concentration of 2 mM dithiothreitol.

**Crude Extract**

To calf liver (1 kg), which had been carefully cleaned of ligaments and divided into small pieces, were added 3,000 ml of 0.05 M Tris-Cl, pH 7.5. The mixture was treated in a Turmix blender for 3 min. The homogenate was centrifuged for 30 min. The precipitate formed was removed by centrifugation for 15 min and the supernatant was adjusted to pH 7.5 with 1.0 M NH₄OH (150 ml).

**Acid Treatment**

The supernatant was adjusted to pH 5.0 with continuous stirring by dropwise addition of ice-cold 1.0 M acetic acid (100 ml). The precipitate formed was removed by centrifugation for 15 min and the supernatant was adjusted to pH 7.5 with 1.0 M NH₄OH (150 ml).

**Heat Treatment**

The neutralized supernatant after acid treatment was diluted to 15 mg of protein per ml with 50 mM Tris-Cl, pH 7.5, and heated to 65° in 1-liter batches under continuous stirring in a boiling water bath. The solution was then rapidly cooled to 4° in an ice bath under continuous stirring. The precipitate was removed by centrifugation for 15 min. To the supernatant was added solid ammonium sulfate to give 90% saturation. The centrifuged precipitate was then dissolved in a small volume of 50 mM Tris-Cl, pH 7.5, and dialyzed overnight against 5 liters of 50 mM Tris-Cl, pH 7.5, and finally desalted on a column of Sephadex G-25 (9 × 30 cm) equilibrated with 10 mM Tris-Cl, pH 7.0.

**DEAE-cellulose Chromatography**

A column of DEAE-cellulose (0 × 18 cm) was equilibrated with 10 mM Tris-Cl, pH 7.0. The desalted ammonium sulfate precipitate was incubated with dithiothreitol and was then adsorbed on the DEAE-cellulose column. The column was eluted with a linear gradient of sodium acetate from 0 to 0.35 M (4,000 ml of each in 10 mM Tris-Cl, pH 7.0). Fractions of 25 ml were collected every 6 min (Fig. 2). Fractions containing thioredoxin activity were combined and concentrated to 20 ml.

**Sephadex G-50 Chromatography**

The concentrated pool from the DEAE-cellulose chromatography was applied to a Sephadex G-50 column (4 × 110 cm) which had been equilibrated with 10 mM Tris-Cl, pH 7.0-0.20 M Tris-Cl, pH 7.5. The neutralized supernatant after acid treatment was diluted to 207

![Fig. 1. Assay of calf liver thioredoxin under standard conditions in the coupled system with insulin (Method 2). The abscissa shows the volume of a solution of homogeneous thioredoxin (8 μg per ml). ▲—▲; of a crude extract (2.34 μg per ml). ○—○; and of a crude extract (2.34 μg per ml) without addition of thioredoxin reductase. ●—●. The conditions of the assay are given under “Experimental Procedure.”](http://www.jbc.org/) Downloaded from http://www.jbc.org/ by guest on August 15, 2017

![Fig. 2. DEAE-cellulose chromatography of ammonium sulfate precipitate. The column (0 × 18 cm) was equilibrated with 10 mM Tris-Cl, pH 7.0 and was eluted with a linear gradient of sodium acetate from 0 to 0.35 M (4,000 ml of each in 10 mM Tris-Cl, pH 7.0). Fractions of 25 ml were collected every 6 min. ●—●, absorbance at 280 nm; ○—○, thioredoxin activity (Method 2). Fractions containing thioredoxin, between the arrows, were pooled. ——, gradient line.](http://www.jbc.org/) Downloaded from http://www.jbc.org/ by guest on August 15, 2017
Fractions of 6 ml were collected every 20 min (Fig. 3). The thioredoxin activity appeared well separated from the main protein peak, which contained thioredoxin reductase activity and could be used for further purification of this enzyme. The thioredoxin-containing fractions were combined and concentrated.

**CM-cellulose Chromatography**

Thioredoxin was incubated with dithiothreitol and equilibrated on a column of Sephadex G-25 with 0.015 M sodium acetate, pH 5.16 (without dithiothreitol). As a final step we used chromatography on a column of CM-cellulose (1 x 12) cm which was equilibrated with the same buffer. The column was eluted with a linear gradient from 15 to 30 mM sodium acetate, pH 5.16 (300 ml of each) (Fig. 4). Thioredoxin-containing fractions were pooled and adjusted to pH 7.0 with 1.0 M Tris base and finally concentrated.

A summary of the purification procedure is given in Table I. Approximately 4000-fold purification with a final yield of 32% was achieved. Most difficulties in the purification arose from the tendency of thioredoxin to aggregate, which resulted in multiple peaks in the chromatograms. This could effectively be prevented by the use of dithiothreitol in the different chromatographic steps.

**Properties of Calf Liver Thioredoxin**

**Purity**—Analytical polyacrylamide gel electrophoresis of the final material showed the presence of a single band (Fig. 5). Determination of the NH₂-terminal amino acid residue by the dansyl method showed the presence of only valine. Both these findings indicated that the protein was homogeneous.

**Molecular Weight**—Determination of the molecular weight of reduced calf liver thioredoxin was performed by analytical gel chromatography on a column of Sephadex G-50. Thioredoxin

![Fig. 3](image-url) Chromatography on a column of Sephadex G-50. The column (4 x 110 cm) was equilibrated with 10 mM Tris-Cl, pH 7.0-0.20 M NaCl. Fractions of 6 ml were collected every 20 min. ● ○, absorbance at 280 nm; ○-○, thioredoxin activity (Method 2).

![Fig. 4](image-url) Chromatography on a column of CM-cellulose (1 x 12 cm). Fractions of 4 ml were collected every 20 min. ● ○, absorbance at 280 nm; ○-○, thioredoxin activity (Method 2). — , sodium acetate.

![Table I](image-url) Purification of calf liver thioredoxin

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein</th>
<th>Thioredoxin activity (Method 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2,650</td>
<td>125,000</td>
<td>27,000 0.22</td>
</tr>
<tr>
<td>pH 5 supernatant</td>
<td>2,350</td>
<td>96,000</td>
<td>22,000 0.39</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>380</td>
<td>15,000</td>
<td>20,000 1.25</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>20</td>
<td>610</td>
<td>18,600 30.5</td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>5.5</td>
<td>18.5</td>
<td>14,200 760</td>
</tr>
<tr>
<td>CM-cellulose</td>
<td>2.5</td>
<td>10.2</td>
<td>8,700 850</td>
</tr>
</tbody>
</table>

* Determined by the Lowry method (13).
* Determined from the absorbance at 280 nm.

![Fig. 5](image-url) Polyacrylamide gel electrophoresis of reduced calf liver thioredoxin (15 μg) after CM-cellulose chromatography. For details see "Experimental Procedure."

![Fig. 6](image-url) Ultraviolet absorption spectra of calf liver thioredoxin. ○-○, in 10 mM Tris-Cl, pH 7.5-0.20 M NaCl-1 mM EDTA; ● ●, in 0.1 M NaOH.
from E. coli was used as a marker with a known molecular weight of 11,700 and calf liver thioredoxin was eluted slightly before the position of this protein. From the result a molecular weight of 12,000 for calf liver thioredoxin was calculated.

**Extinction Coefficient**—The extinction coefficient at 280 nm of the pure calf liver thioredoxin was determined on the basis of dry weight determinations. A value of 14.8 for \((E_{1%})\) was obtained.

**Amino Acid Composition**—The amino acid composition of calf liver thioredoxin is given in Table II. The molecule contained all common amino acids except arginine. For comparison the amino acid composition of E. coli thioredoxin (4) is also included. The most obvious difference was the presence of 4 half-cystine residues in calf liver thioredoxin whereas other thioredoxins contain 2 half-cystine residues (1, 7).

**Ultraviolet Spectra**—The neutral and alkaline spectra of calf liver thioredoxin in the ultraviolet region are given in Fig. 6. The spectra showed no other chromophores than tryptophan and tyrosine. From the extinction coefficient \((E_{1%})\) and a molecular weight of 12,000, a molar absorbance at 280 nm of 8,000 m\(^{-1}\) cm\(^{-1}\) was calculated.

**Peptide Map**—Carboxymethylation of reduced thioredoxin with \(^{14}\)C-labeled iodoacetic acid resulted in incorporation of 3.96 residues of carboxymethylcysteine per molecule of thioredoxin. A peptide map of carboxymethylated thioredoxin after trypsin digestion showed the presence of 12 tryptic-sensitive peptide bonds (12 lysine residues). A peptide map of carboxymethylated thioredoxin after trypsin digestion showed the presence of 12 tryptic-sensitive peptide bonds (12 lysine residues). A peptide map of carboxymethylated thioredoxin after trypsin digestion showed the presence of 12 tryptic-sensitive peptide bonds (12 lysine residues).

**Reoxidation of Reduced Thioredoxin**—Calf liver thioredoxin was isolated in the reduced form and attempts were made to reoxidize the protein in vitro to study the stoichiometry of reduction with NADPH in the presence of thioredoxin reductase. Chemically reduced thioredoxin in 10 mM Tris-Cl, pH 8.0, 200 mM NaCl, 1 mM EDTA, was diluted to a final concentration of \(2.5 \times 10^{-4} \text{ M}\) and exposed to air at room temperature. This treatment resulted in a disappearance of sulfhydryl groups which was matched by the appearance of disulfides reducible by NADPH and thioredoxin reductase. The maximum decrease in sulfhydryl content was obtained after 30 hours when 1.48 sulfhydryl groups per mole of protein had disappeared and 0.78 mole of NADPH per mole of protein was oxidized in vitro enzymatically. These results suggest that in vitro two of the sulfhydryl groups of liver thioredoxin form an oxidation-reduction-active disulfide, but that the reoxidation was incomplete. Attempts to get complete reoxidation of two sulfhydryl groups per mole of thioredoxin were unsuccessful. Furthermore, freezing and thawing solutions of reduced thioredoxin in the absence of dithiothreitol resulted in a slow enzymatic reduction with NADPH and thioredoxin reductase suggesting that mixed disulfides and aggregated thioredoxin were formed.

**Discusion**

The reduced form of calf liver thioredoxin was purified to homogeneity as judged by polyacrylamide gel electrophoresis and the presence of a single NH\(_2\)-terminal amino acid residue. Initial attempts were made to purify the oxidized form of liver thioredoxin without addition of dithiothreitol to buffers in the chromatographic steps. This resulted in the formation of multiple thioredoxin activity peaks and suggested that aggregation of thioredoxin occurred. During the preparation of this manuscript Herrmann and Moore (10) reported the isolation of thioredoxin from rat Novikoff ascites hepatoma. The same aggregation tendency was observed for the Novikoff hepatoma thioredoxin in the absence of a reducing agent. Amino acid analysis of calf liver thioredoxin showed the presence of 4 half-cystine residues and the reoxidation experiments suggested that only 2 of these formed a single oxidation-reduction-active disulfide bridge. It seems highly likely that the additional sulfhydryl groups may readily form mixed disulfides, which would explain the aggregation tendency in vitro of oxidized thioredoxin preparations.

In this study an assay of thioredoxin activity was developed based on the reduction of insulin disulfides by NADPH in the presence of thioredoxin reductase. Attempts to couple the

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**Table II**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Hydrolysis time (hrs)</th>
<th>Assumed integral value</th>
<th>Escherichia coli Thioredoxin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>12 hrs</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>11.86</td>
<td>11.86</td>
<td>12</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.33</td>
<td>1.17</td>
<td>1</td>
</tr>
<tr>
<td>Arginine</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Aspartic acid</td>
<td>9.43</td>
<td>9.13</td>
<td>9</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.00</td>
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<td>3</td>
</tr>
<tr>
<td>Serine</td>
<td>6.51</td>
<td>5.95</td>
<td>6</td>
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<tr>
<td>Glutamic acid</td>
<td>16.85</td>
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</tr>
<tr>
<td>Proline</td>
<td>3.23</td>
<td>3.07</td>
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<tr>
<td>Glycine</td>
<td>5.00</td>
<td>5.06</td>
<td>5</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.14</td>
<td>7.91</td>
<td>8</td>
</tr>
<tr>
<td>Half-cystine*</td>
<td>4.20</td>
<td>4.01</td>
<td>4</td>
</tr>
<tr>
<td>Valine</td>
<td>10.90</td>
<td>10.92</td>
<td>11</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.60</td>
<td>1.69</td>
<td>2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.10</td>
<td>3.91</td>
<td>4</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.35</td>
<td>5.61</td>
<td>6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.87</td>
<td>1.81</td>
<td>2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>9.33</td>
<td>8.29</td>
<td>9</td>
</tr>
<tr>
<td>Tryptophan*</td>
<td>1.0</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>103</td>
</tr>
</tbody>
</table>

* From Holmgren (4).
* Determined as cysteic acid after performic acid oxidation (18), 24 hours of hydrolysis.
* Determined spectrophotometrically according to the method of Benece and Schmid (19).
reaction directly to the reduction of DTNB, as has been used in purification of \textit{E. coli} (1) or yeast thioredoxin (7), were unsuccessful. This result is explained by two observations. First, calf liver thioredoxin contained additional sulfhydryl groups, which probably would be chemically modified by DTNB. Second, the enzyme thioredoxin reductase from calf liver has been observed to reduce DTNB effectively in the presence of NADPH, without the presence of thioredoxin as intermediate electron carrier. This indicates a wider substrate specificity for this mammalian thioredoxin reductase than the corresponding enzymes from \textit{E. coli} (2) or yeast (7).

The properties of the rat Novikoff ascites hepatoma thioredoxin reported by Herrmann and Moore (10) are quite similar to the thioredoxin described in this report. The amino acid composition of the Novikoff tumor thioredoxin, however, showed the presence of 6 half-cystine residues, whereas calf liver thioredoxin had only 4. Experiments are now in progress to determine the amino acid sequence of calf liver thioredoxin. Previously the complete amino acid sequences have been determined for \textit{E. coli} thioredoxin (4) and T4 thioredoxin (27) as well as the active site region of yeast thioredoxin II (28). The amino acid sequence of calf liver thioredoxin would thus enable a comparison of the structures of the same protein from as widely different species as \textit{E. coli} and calf.

REFERENCES

15. Davis, B. J. (1964) \textit{Ann. N. Y. Acad. Sci.} \textbf{121}, 404-427

\footnote{A. Holmgren and N. E. Engström, unpublished results.}
Isolation and Characterization of Calf Liver Thioredoxin
Nils Erik Engström, Arne Holmgren, Agne Larsson and Stefan Söderhäll


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