Purification of a Thioredoxin System from Yeast*

Pedro Gonzalez Porqué,‡ Astor Baldesten, and Peter Reichard§

From the Department of Chemistry II, Karolinska Institutet, Stockholm, Sweden

SUMMARY

In Escherichia coli the two proteins thioredoxin and thioredoxin reductase, together with NADPH, form a hydrogen transport system ("thioredoxin system") which functions during the enzymatic reduction of ribonucleotides. We have now isolated thioredoxin and thioredoxin reductase from baker's yeast.

Yeast thioredoxin was obtained in two forms (thioredoxin I and II). Purified thioredoxin II appeared to be a homogeneous protein with a molecular weight of 12,600. In its oxidized form (thioredoxin-S), it contained a single disulfide bridge which was reduced with NADPH by thioredoxin reductase from yeast (but not from E. coli). The reduced form of thioredoxin (thioredoxin-(SH)2) served as hydrogen donor for E. coli ribonucleotide reductase. The oxidation-reduction potential of the couple thioredoxin-(SH)2-thioredoxin-S2 was -0.24 volt at pH 7. The amino acid composition of yeast thioredoxin II showed considerable differences from that of E. coli thioredoxin.

Yeast thioredoxin reductase is a flavoprotein with FAD as the prosthetic group.

Thioredoxin was identified as the hydrogen donor in the reduction of ribonucleotides to deoxyribonucleotides in Escherichia coli B (1). It is a small protein and, as isolated, contains a single disulfide bridge made up from the 2 half-cystine residues of the molecule. This disulfide bridge is reduced with NADPH by a specific flavoprotein, thioredoxin reductase (2):

\[ \text{Thioredoxin-S} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{reductase}} \text{thioredoxin-(SH)2} + \text{NADP}^+ \]

The dithiol form of thioredoxin is then used for the reduction of the hydroxyl group of the ribotide. This reaction is catalyzed by ribonucleotide reductase:

\[ \text{Ribonucleoside diphosphate} + \text{thioredoxin-(SH)2} \xrightarrow{\text{reductase}} \text{deoxyribonucleoside diphosphate} + \text{thioredoxin-S2} \]

Thioredoxin thus participates in a cyclic oxidoreduction reaction and, together with thioredoxin reductase and NADPH, forms a hydrogen carrier system (thioredoxin system) which makes available the reducing power of NADPH for the reduction of ribonucleotides (3).

Both thioredoxin (4) and thioredoxin reductase (5) were obtained in pure form from E. coli B. Thioredoxin systems were also shown in other sources, such as Lactobacillus leichmannii (6), Novikoff hepatoma (7), regenerating rat liver,1 and phage-infected E. coli (8). They were defined by their ability to act as hydrogen carrier systems during ribonucleotide reduction.

The present investigation was started with a 2-fold purpose: (a) we wished to isolate pure thioredoxin from a source different from E. coli in order to learn something about the general principles involved in the mechanism of action of thioredoxin, and (b) we wanted to investigate the possibility that thioredoxin systems may function as hydrogen carriers in enzymatic reductions other than that of ribonucleotides.

Baker's yeast appeared to be a suitable starting material to obtain sizeable amounts of thioredoxin and its reductase. Furthermore, earlier work by others had shown that in yeast hydrogen carrier systems with properties reminiscent of the thioredoxin system might participate in the reduction of sulfate (9) and methionine sulfoxide (10). In this paper, we describe the preparation and characterization of a highly purified thioredoxin system from baker's yeast. In the accompanying paper (11) we show that this system can function as hydrogen carrier both in the reduction of sulfate and methionine sulfoxide.

EXPERIMENTAL PROCEDURE

Materials

Baker's yeast (Saccharomyces cerevisiae) was a generous gift from Fannyudde AB, Kotebro, Sweden. The yeast was obtained as a 50% suspension in water which was centrifuged for 20 min at 9000 × g and the cell paste was washed with 0.9% NaCl. The cells were then collected by centrifugation and could be stored frozen at -20°C without apparent loss of activity. Before extraction the frozen cells were disintegrated three times by treatment with high pressure (12).

Ribonucleotide reductase after the first DEAE-cellulose step

‡ Awardee of Svensk-Spanska Stiftelsen, Sweden. Present address, Harvard University, The Biological Laboratories, 10 Divinity Avenue, Cambridge, Massachusetts 02138.

§ To whom inquiries should be addressed.

1 A. Larsson and G. Larsson, unpublished results.
Preparative Polyacrylamide Gel Electrophoresis

Spies and Chambers (19).

Biochem. All other reagents were of analytical grade and were obtained from commercial sources.

Methods

Preparative Polyacrylamide Gel Electrophoresis

The runs were done in a Buchler polyacrylamide gel electrophoresis apparatus (Poly-Prep) equipped with a semipermeable rigid glass membrane as described by Thelander (5).

Amino Acid Analyses

The amino acid composition of thioredoxin II was determined according to Spackman, Stein, and Moore (15) with a Spinco model 1203 amino acid analyzer equipped with high sensitivity cuvettes (16). The samples were hydrolyzed under reduced pressure for 24, 60, and 96 hours. Extrapolations to zero time were made for serine. The sulfur-containing amino acids were analyzed after performic acid oxidation as described by Moore (17). Trypsotphan was estimated either spectrophotometrically by the method of Beneze and Schmid (18) or by the method of Spies and Chambers (19).

Peptide Map

A peptide map of a trypsin digest of carboxymethylated (20) thioredoxin II (0.8 mg) was made as described by Holmgren (21) for thioredoxin from E. coli B essentially with the method of Harris and Perham (22).

Protein Determinations

During early stages of purification, protein was determined by the method of Biecher (23). After the Sephadex G-50 step the absorbance at 280 nm of the protein solutions was used; a solution containing 1 mg of protein per ml was assumed to give an absorbance of 1.0 at 280 nm (1 A280 unit). With pure thioredoxin II, we found by refractometry in a Spinco model E analytical ultracentrifuge (24) that 1 A280 unit corresponded to 1.08 mg of the protein.

Ultracentrifugation Analyses

A Spinco model E analytical ultracentrifuge equipped with the RTIC temperature control unit was used. Low speed sedimentation equilibrium measurements were performed as described by Richards, Teller, and Schachman (25). The samples were first equilibrated on a column of Sephadex G-25 with 0.1 M phosphate buffer, pH 7.0. The runs were performed at 19,160 rpm for both thioredoxins and at 8,225 rpm for thioredoxin reductase. The initial protein concentration was determined in the ultracentrifuge with a capillary type synthetic boundary cell. The partial specific volume of thioredoxin II was calculated to be 0.736 ml per g from the amino acid composition (26). A value of 0.724 ml per g was assumed for the partial specific volume of thioredoxin reductase (5).

Thioredoxin Assays

We used three different methods, similar to those used earlier for the assay of E. coli thioredoxin (1).

Method 1—This assay depended on the cross-reaction between reduced yeast thioredoxin and ribonucleotide reductase from E. coli. With an excess of the E. coli enzyme the formation of dCDP from CDP according to Equation 2 of the introductory section depended on the concentration of thioredoxin-(SH)2 in the incubation mixture. All thioredoxin was kept in the reduced form by addition of an excess of dithioerythritol (or an excess of yeast thioredoxin reductase + NADPH).

The reaction mixture contained in a final volume of 0.25 ml: EDTA, 0.25 µmole; ATP, 0.5 µmole; MgCl2, 2 µmoles; 3H-CDP (specific activity, 2000 cpm per µmole) 0.16 µmole; N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid buffer, pH 7.6, 5.0 µmoles; dithioerythritol, 0.5 µmole; ribonucleotide reductase from E. coli, 70 µg; and an unknown amount of thioredoxin. After incubation for 30 min at 25° the reaction was terminated by addition of 1 ml of 3 HClO4 and the amount of dCDP formed was determined. One unit of thioredoxin activity is defined as the amount of thioredoxin required to allow the formation of 1 µmole of dCDP under the above conditions.

Specific activity is defined as units per mg of protein.

Method 2—Here the reaction represented by Equation 1 of the introductory section was carried out in the presence of an excess of 5,5'-dithiobis(2-nitrobenzoic acid) which reacted with the reduced thioredoxin to form a colored product and to regenerate thioredoxin-S-S. Since thioredoxin is recycled, the rate of color formation was a relative measure of the amount of thioredoxin present.

Each of two cuvettes contained: 100 µmoles of potassium phosphate buffer (pH 7.0), 10 µmoles of EDTA, 0.1 µmole of NADPH, and about 30 µg of thioredoxin reductase from yeast (after DEAE-cellulose chromatography). Thioredoxin was added to one of the cuvettes and the volumes of both cuvettes were adjusted to 1 ml with water. Then 0.2 ml of a fresh solution of 5,5'-dithiobis(2-nitrobenzoic acid) (4 mg of 5,5'-dithiobis(2-nitrobenzoic acid) per ml of absolute ethanol + 9 ml of Tris, 0.5 M, pH 8.0) was added to each cuvette. The absorbance at 412 nm of the thioredoxin-containing cuvette was read at 1-min intervals with the other cuvette as a blank. Usually three different determinations could be made simultaneously. One unit of thioredoxin is defined as the amount of protein which at room temperature under the above conditions gave an increase in the absorbance at 412 nm of 1 per min.

Method 3—Since Reaction 1 at neutral pH goes to completion the amount of thioredoxin-S-S present in a sample can be determined by measuring the total amount of NADPH which disappears during the reaction.

Each of two cuvettes contained: 0.1 µmole of NADPH, 120 µmoles of potassium phosphate buffer (pH 7.0), and 10 µmoles of EDTA in a final volume of 1.2 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 µm, 1 to 2 µg of thioredoxin reductase (after gel electrophoresis) were added to each cuvette, and the apparent increase in the absorbance at 340 µm was recorded at different time intervals. A constant reading should be obtained after 2 min. From the difference between

Downloaded from www.jbc.org/ by guest on March 24, 2020
Comparison of Methods

Method 1 could be used at all stages of purification. Its major drawback was the amount of time involved. Method 2 is not very accurate and could not be used during the early stages of purification. It is very rapid and can be made very sensitive. It is the method of choice for locating thioredoxin during column chromatography. Method 3 is the only method which gives the absolute amount of thioredoxin present in a sample. However, reliable values are only obtained with quite pure samples of thioredoxin, since other disulfide-containing compounds may give rise to recycling of thioredoxin and thus interfere with the assay.

RESULTS

Purification of Thioredoxin

Unless stated otherwise, all manipulations were carried out around 4°C; centrifugations were performed at 15,000 x g. All Tris buffers were neutralized with HCl and the molarities refer to the Tris ion. All buffers contained 0.001 M EDTA.

Extraction—Disintegrated yeast (2176 g) was stirred with 6500 ml of 0.05 M Tris, pH 7.5, for 30 min. Insoluble material was removed by centrifugation for 20 min.

Streptomycin Sulfate Precipitation—To the supernatant solution (7350 ml) 441 ml of a 20% solution of streptomycin sulfate were added slowly (about 1 hour) with vigorous stirring. After 1 additional hour the precipitate was removed by centrifugation for 10 min.

Ammonium Sulfate Precipitation and Acid Treatment—To the supernatant solution (7500 ml) 441 ml of a 20% solution of streptomycin sulfate were added slowly (about 1 hour) with vigorous stirring. After 1 additional hour the precipitate was removed by centrifugation for 10 min.

Ammonium Sulfate Precipitation and Acid Treatment—To the supernatant solution (7500 ml) 441 ml of a 20% solution of streptomycin sulfate were added slowly (about 1 hour) with vigorous stirring. After 1 additional hour the precipitate was removed by centrifugation for 10 min.

Heat Treatment—The supernatant solution after acid treatment (1725 ml) was diluted with 0.05 M Tris, pH 7.5, to a final
protein concentration of 0.28 units per ml, and heated in batches of about 2 liters with vigorous stirring in a boiling water bath. When the solution had reached a temperature of 60° it was cooled in an ice-NaCl bath (−10°). The precipitate was discarded after centrifugation.

Chromatography on Sephadex G-50—Thioredoxin was precipitated from the supernatant solution from the previous step (4000 ml) by addition of ammonium sulfate (0.612 g per ml); the precipitate was allowed to settle overnight, collected by centrifugation for 90 min, dissolved in about 300 ml of 0.01 M ammonium acetate (pH 8.6), and chromatographed on a column of Sephadex G-50 (11 × 140 cm) equilibrated with the same buffer. Elution (70- to 80-ml fractions) was performed at a rate of 350 ml per hour with the same buffer. Thioredoxin activity appeared in the latter part of the chromatogram (Fig. 1) well separated from the main protein peak which contained thioredoxin reductase activity. The fractions containing thioredoxin (45 to 60) were pooled.

First DEAE-cellulose Chromatography—A column of DEAE-cellulose (5 × 24 cm) was washed first with 3 liters of 0.2 M potassium buffer, pH 7.0, on a short Sephadex column with the “upper gel buffer” (5). When the solution had reached a temperature of 60° it was cooled in an ice-NaCl bath (−10°). The precipitate was discarded after centrifugation.

Second DEAE-cellulose Chromatography—Both thioredoxins were rechromatographed on columns of DEAE-cellulose (3 × 30 cm, 5 ml of DEAE-cellulose per mg of protein). Both thioredoxin activities coincided with the main protein peaks appearing at acetate concentrations of 0.046 M (thioredoxin I) and 0.057 M (thioredoxin II). The active fractions were pooled and lyophilized as described above. Before gel electrophoresis both thioredoxins were equilibrated on short Sephadex columns with the “upper gel buffer” (5).

Preparative Polyacrylamide Gel Electrophoresis—Thioredoxin I (16 mg) or thioredoxin II (12 mg) from the previous steps were introduced in about 10 ml of the upper gel buffer containing 12% sucrose and layered carefully on top of the gel. The final separation was done at 85 mA which gave a potential of about 1000 volts over the apparatus. Seven-milliliter fractions were collected at 4-min intervals and analyzed for protein and thioredoxin activity (Fig. 3, A and B). The fractions containing thioredoxin were pooled and lyophilized with mannitol. Finally, thioredoxin was equilibrated with 0.1 M potassium buffer, pH 7.0, on a short column of Sephadex G-25.

A summary of the complete purification procedure is given in Table I. During the purification the largest difficulties arose from the necessity of reducing the volumes of the very dilute solutions obtained after the chromatographic steps. Both thioredoxins showed a tendency to aggregate, particularly at acid pH values. This difficulty, which was more pronounced with thioredoxin I, was in part prevented by using mannitol during the lyophilizations.

Properties of Thioredoxins

Evidence is presented below that thioredoxin II was obtained as a homogeneous protein, while our best preparations of thioredoxin I still contained some minor impurities. For this reason, a more detailed characterization is only given for thioredoxin II.

Molecular Weight—Sedimentation equilibrium runs were done with both thioredoxins and a plot of ln c against x² for thioredoxin II is given in Fig. 4. A linear relationship was found over the whole cell, indicating that the preparation was homogeneous

![Fig. 4. Molecular weight determinations of thioredoxin II by low speed sedimentation equilibrium. The ordinate gives the In c against x² for thioredoxin II is given in Fig. 4. A linear relationship was found over the whole cell, indicating that the preparation was homogeneous.](http://www.jbc.org/)
Amino acid composition of thioredoxin II

| Amino acid | Hydrolysis for | Calculated |
|------------|----------------|------------|
|            | 20 hrs | 60 hrs | 96 hrs | residues/molecule |
| Lysine     | 10.8   | 10.0   | 10.2   | 10-11             |
| Histidine  | 0.2    | 0.2    | 0.2    |                   |
| Arginine   | 1.5    | 1.5    | 1.5    | 1-2               |
| Aspartic acid | 10.3  | 10.4   | 10.3   | 10                |
| Threonine  | 4.0    | 3.9    | 3.9    | 4                 |
| Serine     | 8.7    | 7.6    | 7.6    | 9b                |
| Glutamic acid | 12.7  | 13.0   | 12.7   | 13                |
| Proline    | 4.3    | 4.1    | 4.2    | 4                 |
| Glycine    | 6.0    | 6.2    | 6.0    | 6                 |
| Alanine    | 15.9   | 15.7   | 15.7   | 16                |
| Valine     | 11.2   | 11.2   | 11.3   | 11                |
| Isoleucine | 5.1    | 5.1    | 5.2    | 5                 |
| Leucine    | 6.0    | 5.8    | 5.8    | 6                 |
| Tyrosine   | 4.0    | 4.4    | 4.0    | 4                 |
| Phenylalanine | 5.1  | 4.7    | 5.0    | 5                 |
| Half-cystine | 1.8   | 1.8    | 2c     |                   |
| Methionine | 1.9    | 2.3    | 2e     |                   |
| Tryptophan | 1.8    |        | 1d     |                   |

* The results are expressed as residues per molecule, assuming a molecular weight of 12,600.

** Extrapolated to zero time hydrolysis.

\(^{4}\) Determined after performic acid oxidation.

\(^{5}\) Determined according to Spies and Chambers (19) or Bencze and Schmid (18).

** Purification of Thioredoxin Reductase

This enzyme was purified together with thioredoxin up to the Sephadex G-50 step. At this point (cf. Fig. 1) thioredoxin reductase was eluted close to the void volume. For further purification, the materials from two chromatograms were pooled, precipitated with ammonium sulfate (0.612 g per ml), and centrifuged. The precipitate was dissolved in a minimal volume of 0.02 M potassium phosphate buffer, pH 7.0, and passed through a column of Sephadex G-25 equilibrated with the same buffer.

DEAE-cellulose Chromatography—A column of DEAE-cellulose (6 × 18 cm) was equilibrated with potassium phosphate buffer, pH 7.0 (5 liters of 0.2 M + 5 liters of 0.02 M). The thioredoxin reductase solution (470 ml) was then adsorbed to the column and chromatographed with a linear gradient of phosphate buffer, pH 7.0 (0.05 M to 0.16 M, 3 liters of each). Elution was carried out at a rate of about 150 ml per hour. The peak of thioredoxin reductase activity appeared around 0.11 M phosphate. The active fractions were pooled (1000 ml) and concentrated by ultrafiltration to a final volume of 2 to 3 ml.

Sephadex G-100 Chromatography—The concentrated solution was diluted 2-fold with water and chromatographed on a column of Sephadex G-100 (3 × 175 cm), equilibrated with 0.05 M Tris buffer, pH 7.0. Four-milliliter fractions were collected at 20-min intervals and analyzed for protein and enzyme activity with respect to molecular weight. On the other hand, a similar experiment with thioredoxin I showed a quite sudden increase of the slope at the bottom of the cell, indicating contamination with heavy material.

The partial specific volume of thioredoxin II, calculated (25) from the amino acid composition (see below), was 0.736 ml per g. With this value a molecular weight of 12,600 could be calculated (24).

Amino Acid Composition and Peptide Map—The amino acid composition of thioredoxin II is given in Table II. The calculations were based on a molecular weight of 12,600. The molecule contained 1 single residue of tryptophan and 1 or 2 residues of arginine; 2 half-cystines and 2 methionines were found.

Thioredoxin II contained a total of 11 to 13 trypsin-sensitive peptide bonds (10 to 11 lysine + one to two arginine). A peptide map after trypsin digestion showed the presence of 12 ninhydrin-positive spots (Fig. 5).

Spectrum—Spectra of thioredoxin II at neutral and alkaline pH are given in Fig. 6. The presence of chromophores other than tryptophan and tyrosine is not indicated. Similar spectra were found with thioredoxin I but are not shown here.
FIG. 7. Sephadex G-100 chromatography of thioredoxin reductase. ●, absorbance at 280 nm; ○—○, thioredoxin reductase activity.

FIG. 8. Preparative polyacrylamide gel electrophoresis of thioredoxin reductase. ●, absorbance at 280 nm; ○—○, thioredoxin reductase activity.

The active fractions (69 ml) were pooled and concentrated to 3 ml by ultrafiltration.

Preparative Polyacrylamide Gel Electrophoresis—This step was carried out as described for thioredoxin. The resulting purification is illustrated by Fig. 8. The active fractions were pooled and concentrated by ultrafiltration to a final volume of 2 ml. A summary of the latter part of the purification procedure is given in Table III.

**Properties of Thioredoxin Reductase**

Spectrum and FAD Content—The spectrum of thioredoxin reductase in 0.1 M phosphate buffer, pH 7.0, is shown in Fig. 9 (Curve A). It had three maxima around 276, 380, and 460 nm and thus resembled the spectrum of the E. coli enzyme. When a solution of the yeast reductase was boiled for 5 min, the spectrum of the supernatant solution after centrifugation showed two maxima at 373 and 450 nm, respectively (Fig. 9, Curve B). This spectrum was then similar to that of free FAD (Fig. 9, Curve C). Furthermore, after boiling of the enzyme, FAD was identified directly in the supernatant solution by thin layer chromatography on polyethyleneimine cellulose (5, 27).

The amount of FAD in the enzyme could be estimated from the absorbance at 450 nm of the boiled enzyme solution and the known molar extinction coefficient (11.3 x 10³) of the nucleotide. According to such a calculation 1 A₂₆₀ unit of enzyme corresponded to about 7 mmoles of FAD.

**Ultracentrifugation**—In sedimentation velocity experiments thioredoxin reductase (4 mg per ml) sedimented as a single peak with an s₂₀,₅₀ value of 5.0 S. On low speed sedimentation equilibrium centrifugation it was apparent, however, that the enzyme was not homogeneous, since plots of ln c against r² showed an upward curvature. An approximate molecular weight of the enzyme could be obtained from the data by using the two extreme slopes of the plot at the meniscus and the bottom of the cell. In this way limiting values of 64,000 and 80,000 were obtained.

**Properties of Thioredoxin System**

**Stoichiometry**—The stoichiometry of reaction (1) was established with both thioredoxin I and II by comparing the appearance of -SH groups with the disappearance of NADPH.

In a first experiment, 0.149 mg of thioredoxin I was incubated with NADPH and thioredoxin reductase under the conditions of Method 3. From the decrease in absorbance at 340 nm, it could be calculated that 10.8 mmoles of NADPH had been oxidized when the reaction had gone to completion. At this point the sample was boiled for 1 min and the amount of —SH groups was determined with 5,5'-dithiobis(2-nitrobenzoic acid). A value of 20.7 mmoles was obtained.
**FIG. 10.** pH optimum of thioredoxin reductase. Experiments were done with 0.182 μmole of thioredoxin I (open symbols) and 0.168 μmole of thioredoxin II (closed symbols), respectively, in acetate (Δ, ▲), phosphate (⊙, ●), or Tris (□, ■) buffers. Method 2 was used.

**FIG. 11.** Lineweaver-Burk plot for thioredoxins. The experiments were done with Method 2 with 0.002 A_{280} unit of thioredoxin reductase (after gel electrophoresis) and varying amounts of thioredoxin I (O---O) or II (O--O). In a similar experiment, 0.143 mg of thioredoxin II consumed 11.8 μmole of NADPH and produced 21.4 μmole of -SH groups.

In both experiments thus almost 2 moles of SH were produced for each mole of NADPH consumed. The slightly low figures are probably explained by reoxidation of some -SH groups during the boiling process.

From the above data we can calculate that 13.700 g of thioredoxin I and 12.200 g of thioredoxin II, respectively, were required for the oxidation of 1 mole of NADPH.

**pH Optimum**—Fig. 10 shows that the reduction of both thioredoxin I and II had a pH optimum of about 6.5. The reaction appeared to be inhibited in Tris buffers as compared to acetate or phosphate buffers.

**K_m Values for Thioredoxin**—Lineweaver-Burk plots for both thioredoxins at saturating concentrations of NADPH are given in Fig. 11. K_m values of 2.8 × 10^{-4} M and 2.3 × 10^{-4} M were obtained for thioredoxins I and II, respectively.

**Equilibrium of Reaction 1**—The determination of the equilibrium constant

\[
K_{eq} = \frac{(\text{thioredoxin}-(\text{SH})_2)(\text{NADP}^+)}{(\text{thioredoxin}-\text{S}_2)(\text{NADPH})(\text{H}^+)}
\]

was made with yeast thioredoxin II by a procedure used previously for *E. coli* thioredoxin (2). Thioredoxin was incubated at pH 7.85 and 8.8, respectively, with a small excess of NADPH and thioredoxin reductase, and the time-dependent decrease in the absorbance at 340 μm was recorded. When equilibrium had been reached a measured excess of NADP was added. This resulted in the establishment of new equilibria (Fig. 12). From the known starting concentrations of the reactants, the stoichiometry of Reaction 1, and the changes in absorbance at 310 μm, the different equilibrium concentrations of the components could be determined and used for the calculation of K_{eq} (2). In this way, values of 2.1 × 10^3 (pH 7.85) and 4.0 × 10^3 (pH 8.8) were obtained. From the average value of K_{eq} = 3 × 10^3 and the known oxidation-reduction potential of the NADPH-NADP^+ couple \((-0.305 \text{ volt at pH 7 and 20°C})\), the oxidation-reduction potential for the couple thioredoxin-(SH)_2-thioredoxin-S_2 from yeast could be calculated to be \(-0.24 \text{ volt}\). This value is...
slightly more positive than the corresponding value for the thioredoxin couple from \textit{E. coli} (-0.26 volt).

**Specificity of Yeast Thioredoxin Reductase**—The enzyme showed a high specificity for the reduction of the homologous thioredoxins, and did not react with thioredoxins from \textit{E. coli} or \textit{T4}-infected \textit{E. coli} (8).

**Comparison of Efficiency of Thioredoxins as Hydrogen Donors**—Both yeast thioredoxins served as hydrogen donors for the \textit{E. coli} ribonucleotide reductase and this was in fact the basis for the assay used during purification. A comparison between the effects of thioredoxins from \textit{E. coli} and from yeast is shown in Fig. 13. It is clear that on a molar basis \textit{E. coli} thioredoxin was most efficient and that yeast thioredoxin I was more active than thioredoxin II.

**DISCUSSION**

Our experiments show the existence of a thioredoxin system in yeast similar to the one described earlier for \textit{E. coli}. In crude extracts, yeast thioredoxin and thioredoxin reductase were identified by their capacity to function as hydrogen carriers in the reduction of ribonucleotides catalyzed by \textit{E. coli} ribonucleotide reductase. This also formed the basis for the assay used during the purification of the two proteins. When highly purified preparations of thioredoxin and its reductase became available, it was possible to characterize them by chemical means and to show that their properties closely corresponded to those of the components of \textit{E. coli} system. The main characteristic of thioredoxin was the presence of the oxidation-reduction couple ($\text{SH}_2$),$\text{S}_2$ formed by 2 half-cystine residues. Thioredoxin reductase was characterized as an FAD-containing enzyme which specifically catalyzed the reduction of the oxidized form of thioredoxin by NADPH.

On chromatography on DEAE-cellulose yeast thioredoxin separated into two species which were labeled I and II. Thioredoxin II was obtained in essentially pure form as judged from ultracentrifugation data and from the excellent agreement between the molecular weights determined either by sedimentation equilibrium centrifugation (12,600) or from the stoichiometry of Reaction 1 (12,200). The size of yeast thioredoxin was thus very similar to that of \textit{E. coli} thioredoxin, which has a molecular weight of 11,700 (4). On the other hand, the amino acid compositions and the peptide maps after tryptic digestion of yeast and \textit{E. coli} thioredoxins were quite different. The structural dissimilarities are also reflected in the different behavior during purification (yeast thioredoxin is clearly a less acidic protein than \textit{E. coli} thioredoxin), the difference in oxidation-reduction potentials of the ($\text{SH}_2$),$\text{S}_2$ couples, and the high specificity of the two thioredoxin reductases for their homologous thioredoxins.

Thioredoxin I had properties very similar to those of thioredoxin II. It has not yet been obtained in completely pure form and it is therefore not possible to understand the molecular basis for the occurrence of the two forms.

Thioredoxin reductase from yeast resembled the enzyme from \textit{E. coli} in being a flavoprotein with FAD as the prosthetic group. Since the protein was not obtained in a completely homogeneous form it was not possible to determine the stoichiometry of the FAD content. Our best preparations of yeast thioredoxin reductase contain between 0.5 and 1 mole of FAD per mole of protein. \textit{E. coli} thioredoxin reductase contains 2 moles of FAD per mole of protein.

The experiments described in this paper show the ability of yeast thioredoxin—($\text{SH}_2$)$\text{S}_2$ to function as hydrogen donor for the \textit{E. coli} ribonucleotide reductase. Preliminary experiments with a crude ribonucleotide reductase from yeast showed that yeast thioredoxin also functions as hydrogen donor for this enzyme. However, this does not appear to be the only function of yeast thioredoxin. In the following paper (11), we present evidence for its participation in other reductive processes in yeast.

**REFERENCES**

1. Laubret, T. C., Moore, E. C., and Reichard, P., \textit{J. Biol. Chem.}, \textbf{239}, 3436 (1964).
2. Moore, E. C., Reichard, P., and Thelander, L., \textit{J. Biol. Chem.}, \textbf{238}, 3445 (1964).
3. Reichard, P., \textit{The biosynthesis of deoxyribose}, Ciba Lectures, John Wiley and Sons, New York, 1968.
4. Holmgren, A., \textit{Eur. J. Biochem.}, \textbf{6}, 475 (1968).
5. Thelander, L., \textit{J. Biol. Chem.}, \textbf{242}, 852 (1967).
6. Oer, M. D., and Ytvol, E., \textit{Biochem. Biophys. Res. Commun.}, \textbf{25}, 105 (1966).
7. Moore, E. C., \textit{Biochem. Biophys. Res. Commun.}, \textbf{29}, 264 (1967).
8. Berglund, O., \textit{J. Biol. Chem.}, \textbf{244}, 4299 (1969).
9. Wilson, L. G., Asahi, T., and Bandurski, R. S., \textit{J. Biol. Chem.}, \textbf{236}, 1822 (1961).
10. Black, S., Harte, M., Hudson, B., and Wartofsky, L., \textit{J. Biol. Chem.}, \textbf{235}, 2910 (1960).
11. Goncalves-Ponquay, P., Balnstein, A., and Reichard, P., \textit{J. Biol. Chem.}, \textbf{245}, 2371 (1970).
12. Edsoll, T., \textit{J. Biochem. Microbiol. Techn. Engin.}, \textbf{3}, 151 (1961).
13. Brown, N. C., Caneklakis, Z. N., Lundin, B., Reichard, P., and Thelander, L., \textit{Eur. J. Biochem.}, \textbf{9}, 561 (1969).
14. Peterson, E. A., and Sober, H. A., \textit{J. Amer. Chem. Soc.}, \textbf{78}, 753 (1956).
15. Speckman, D. H., Stein, W. H., and Moore, S., \textit{Anal. Chem.}, \textbf{30}, 1190 (1958).
16. Technical Bulletin A-BT-OlOA, Spinco Division, Beckman Instruments, Inc., Palo Alto, 1965.
17. Moore, S., \textit{Anal. Chem.}, \textbf{36}, 235 (1965).
18. Bendig, W. J., and Schmitt, R., \textit{Anal. Chem.}, \textbf{28}, 1193 (1957).
19. Spies, J. R., and Chambers, D. S., \textit{Anal. Chem.}, \textbf{21}, 1249 (1949).
20. Crestfield, A. M., Moore, S., and Stein, W. H., \textit{J. Biol. Chem.}, \textbf{236}, 622 (1963).
21. Holmgren, A., \textit{Eur. J. Biochem.}, \textbf{5}, 359 (1968).
22. Harris, J. L., and Pihrm, K. N., \textit{J. Mol. Biol.}, \textbf{13}, 876 (1965).
23. Boccher, T., \textit{Biochem. Biophys. Acta}, \textbf{1}, 292 (1947).
24. Goldberg, M. E., Crichtson, T. E., Baldwin, R. L., and Yanofsky, C., \textit{J. Mol. Biol.}, \textbf{21}, 71 (1966).
25. Richards, E. G., Tellier, D. C., and Schachman, H. K., \textit{Biochemistry}, \textbf{7}, 1054 (1968).
26. Cohn, E. J., and Edsall, J. T., \textit{Proteins, amino acids and peptides as dipolar ions}, Reinhold Publishing Corporation, New York, 1943, p. 370.
27. Randerath, K., \textit{Biochim. Biophys. Acta}, \textbf{61}, 592 (1962).
Purification of a Thioredoxin System from Yeast
Pedro Gonzalez Porqué, Astor Baldesten and Peter Reichard

J. Biol. Chem. 1970, 245:2363-2370.

Access the most updated version of this article at http://www.jbc.org/content/245/9/2363

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/245/9/2363.full.html#ref-list-1