The Involvement of the Thioredoxin System in the Reduction of Methionine Sulfoxide and Sulfate

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SUMMARY

Earlier work by others had established the occurrence of two complex enzyme systems in yeast which catalyze the reductions of methionine sulfoxide and sulfate, respectively. We now show that thioredoxin and thioredoxin reductase from yeast can function as hydrogen carriers in both types of reactions and it is suggested that they form part of both enzyme systems.

Black et al. (1) demonstrated that in yeast the enzymatic reduction of methionine sulfoxide to methionine by NADPH requires the combined action of three protein fractions (Enzymes I, II, and III). Furthermore, Enzymes I and II together reduced different disulfides (e.g., oxidized lipoic acid, glutathione, and other peptides) with NADPH.

Wilson, Asahi, and Bandurski (2) and Asahi, Bandurski, and Wilson (3) found a similar situation with respect to the enzymatic reduction of sulfate in yeast. The reduction of "active sulfate" (3'-phosphoadenosine 5'-phosphosulfate) by NADPH required three separated protein fractions (Enzymes A and B and Fraction C), two of which (Enzyme A and Fraction C) could function as a disulfide reductase.

Work in this laboratory (4, 5) showed that the thioredoxin system from E. coli B (thioredoxin + thioredoxin reductase + NADPH) could function as a disulfide reductase and suggested the possibility that the thioredoxin system may be involved in the reductions of both methionine sulfoxide and of sulfate.

In the preceding communication (6), we described the preparation of a thioredoxin system from yeast. Here we show that this system together with Enzyme III (1) or Enzyme B (2, 3) can participate in the reduction of methionine sulfoxide and sulfate, respectively.

MATERIALS AND METHODS

DEAE-cellulose was prepared according to Peterson and Sober (7). Sephadex of different grades was purchased from Pharmacia. * This work was supported by grants to P. Reichard from The Damo Foundation, Magnus Bergvalls Foundation, and Gustaf och Tyra Svenssons Minne.

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The recovery of carrier sulfite was in most cases better than 80%. Sulfite were used for the determination of radioactivity and sulfite (12). Sulfite were recovered in NaOH in the center well. Aliquots addition of acid from the side arm and volatile % and carrier and with the same buffer. The final product (17 mg) had a specific activity (1) of 0.032 unit per mg.

Enzymes had been removed (2) and %S-labeled PAPS was therefore used as substrate. Part A of Fig. 3 shows the dependence of sulfite formation on the amount of thioredoxin reductase (O—O). In the latter experiments 4 μmols of thioredoxin were added to each tube.

Analysis. For further details the legend to Fig. 5 should be consulted.

Protein Determinations During the early stages of purification of Enzyme III and Enzyme B a turbidimetric method (13) was used. With the purified enzymes we assumed a value of 1.0 for the absorbance at 280 nm of a solution containing 1 mg of protein per ml.

RESULTS

Enzymatic Reduction of Sulfate

Crude Extract—When the reduction of sulfate was studied with increasing amounts of crude yeast extract the S-shaped curve of Fig. 1 was observed. The nonlinearity indicated the operation of a multienzyme system, confirming the findings of earlier workers (2, 3).

The addition of increasing amounts of yeast thioredoxin strongly stimulated the formation of sulfite from sulfate, while addition of thioredoxin reductase showed little effect (Fig. 2). These experiments gave the first indication that thioredoxin and thioredoxin reductase might participate as a hydrogen donor during the reduction of sulfate.

Purified PAPS Reductase—Fig. 3 shows experiments with the purified enzyme. During purification, the sulfate-activating enzymes had been removed (2) and %S-labeled PAPS was therefore used as substrate. Part A of Fig. 3 shows the dependence of sulfite formation on the amount of PAPS reductase added. In these experiments, thioredoxin and thioredoxin reductase were added to the incubation mixtures. Part B of Fig. 3 shows the dependence of sulfite formation on the amount of thioredoxin added (in the presence of thioredoxin reductase) and Part C gives the results with increasing amounts of thioredoxin reductase (in the presence of thioredoxin). With the purified PAPS reductase the enzymatic formation of sulfite was clearly completely dependent on the simultaneous presence of thioredoxin and its reductase.

In these experiments the amount of sulfite formed during the

![Fig. 1. Sulfite formation in crude extracts. The incubation mixture contained the following reagents (in micromoles) in a final volume of 1 ml: ATP, 2; %S-Na₂SO₄ (6.3 × 10⁵ cpm per μmole), 5; NADP, 0.06; MgCl₂, 5; potassium phosphate (pH 7.0), 20; EDTA, 1.5; and Na₂SO₄, 5. Glucose 6-phosphate dehydrogenase (0.5 Kornberg unit) and protein from yeast acetone powder in the amounts indicated on the abscissa were also added. Incubation was carried out for 1 hour at 37°C.](image1)

![Fig. 2. Effects of thioredoxin and thioredoxin reductase on sulfite formation. The general incubation conditions are given in Fig. 1. Two milligrams of protein from the crude extract were used. The experiments show the effects of increasing amounts of thioredoxin (O—O) or thioredoxin reductase (O—O). In the latter experiments 4 μmols of thioredoxin were added to each tube.](image2)
Sulfate reductase ($\alpha$)
Thioredoxin (MX105)
Thioredoxin reductase ($\beta$)

**FIG. 3.** Sulfite formation with purified PAPS reductase. A, dependence on increasing amounts of enzyme. Incubation mixtures contained the following reagents (in micromoles) in a final volume of 0.75 ml: Tris (pH 7.5), 25; EDTA, 0.7; MgCl$_2$, 2.5; NADPH, 0.5; $^{35}$S-PAPS (5 x 10$^6$ cpm per pmole), 0.11; Na$_2$SO$_4$, 2.5; and thioredoxin, 0.0044. Thioredoxin reductase, 9 $\mu$g, was also added. B, dependence on thioredoxin. General conditions were as in A. All tubes contained 60 $\mu$g of PAPS reductase. C, dependence on thioredoxin reductase. General conditions were as in B. All tubes contained 4.4 nmol of thioredoxin.

**FIG. 4.** Time curve of sulfite formation. Incubation was carried out in a final volume of 1.5 ml containing the following reagents (in micromoles): Tris (pH 7.5), 50; EDTA, 1.4; MgCl$_2$, 5; NADPH, 1.0; $^{35}$S-PAPS, 0.22, and thioredoxin, 0.007. Thioredoxin reductase, 17 $\mu$g, and PAPS reductase, 0.99 mg, were also added. At different time intervals 0.2 ml was removed from the incubation mixture and used for the assays.

2-hour incubation period did not exceed the amount of thioredoxin added to the system. In Fig. 4 a time curve of the reaction is given, in which a larger amount of PAPS reductase was used. After 5 hours, 21 nmol of sulfite was formed in the presence of 7 nmol of thioredoxin. This experiment indicates that thioredoxin has a catalytic function during the reduction of PAPS.

**Enzymatic Reduction of Methionine Sulfoxide**

With purified methionine sulfoxide reductase the formation of methionine was completely dependent on the simultaneous addition of thioredoxin and thioredoxin reductase (Fig. 5, A to C).

Furthermore, the experiments clearly show that thioredoxin had a catalytic function. The amount of methionine formed during a 10-min period in some cases exceeded the amount of thioredoxin added by more than a factor of 10. Fig. 5, A and

**FIG. 5.** Reduction of methionine sulfoxide. A, dependence on methionine sulfoxide reductase. Incubation was carried out in microcuvettes, each containing the following reagents (in micromoles) in a final volume of 0.14 ml: Tris (pH 7.7), 7.5; methionine sulfoxide, 1; and NADPH, 0.05. Each cuvette also contained serum albumin, 0.23 mg, varying amounts of methionine sulfoxide reductase, and either 1.2 nmol of yeast thioredoxin and 1.7 $\mu$g of yeast thioredoxin reductase (O-O) or 2.4 nmol of E. coli thioredoxin and 0.8 $\mu$g of E. coli thioredoxin reductase (■-■). Incubation was carried out at room temperature and the decrease in the absorbance at 340 nm was recorded with a Zeiss M4QII spectrophotometer. The reaction was started by the addition of methionine sulfoxide. Essentially no oxidation of NADPH occurred in the absence of methionine sulfoxide. The ordinate gives the amount of NADPH oxidized during 10 min. B, dependence on thioredoxin. The general conditions were as described for A. Each cuvette contained 0.23 mg of methionine sulfoxide reductase and either 1.7 $\mu$g of yeast thioredoxin reductase (O-O) or 0.8 $\mu$g of E. coli thioredoxin reductase (■-■). C, dependence on thioredoxin reductase. The general conditions were as described for A. Each cuvette contained 0.23 mg of methionine sulfoxide reductase and 1.2 nmol of yeast thioredoxin.

**FIG. 6.** General scheme for the participation of the thioredoxin system in biological reductions.

B, also shows two experiments (filled circles) with thioredoxin and thioredoxin reductase from E. coli. It is apparent that yeast methionine sulfoxide reductase could use the heterologous reduced thioredoxin.

**DISCUSSION**

Thioredoxin and thioredoxin reductase were originally discovered in E. coli as components of a hydrogen carrier system which participates in the complex enzyme system responsible for the reduction of ribonucleotides to deoxyribonucleotides (4). In the preceding paper (6), we described the purification of thioredoxin and thioredoxin reductase from yeast. The two proteins
were defined by their capacity to substitute for the E. coli thioredoxin system during ribonucleotide reduction.

The results presented in this paper clearly establish that thioredoxin and its reductase can function as hydrogen carriers in two further processes: the reduction of sulfate and that of methionine sulfoxide. In both cases, earlier work (1-3) had implicated the participation of proteins with properties similar to thioredoxin and thioredoxin reductase in the reaction sequences.

It appears that the thioredoxin system may have a quite general function in several reductions. This is schematically depicted in Fig. 6. There, reduced thioredoxin serves as the hydrogen donor in the reduction of an oxidized substrate which may be a ribonucleotide, sulfate, or methionine sulfoxide; each reaction is catalyzed by a specific reductase. In a formal sense, the three reactions involve the removal of an oxygen from the substrate and the thioredoxin system functions by making available the reducing power of NADPH for this purpose. It seems possible that other reductases than those implicated above may be coupled to the thioredoxin system. The capacity of thioredoxin-(SH)$_2$ to reduce different disulfides (1, 5) should also be recalled. This latter reaction is nonenzymatic.

Finally, we want to point out that our experiments do not definitely establish that the thioredoxin system in vivo participates during the reduction of sulfate or methionine sulfoxide. Decisive proof for this would require a detailed characterization of mutants blocked in the reaction sequences. With respect to the reduction of sulfate, such mutants appear already to be available (14).

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