Thioredoxin reductase is a flavo-enzyme that catalyzes the reduction, by NADPH, of the active site disulfide bond of thioredoxin, with an overall mechanism similar to that of glutathione reductase (1, 2). The electron transfer proceeds from NADPH, via the flavin, to the putative active site, which then exchanges hydrogens with the oxidized disulfide of the substrate. The thioredoxin system has been implicated in a variety of functions, including DNA synthesis (by serving as a hydrogen donor for ribonucleotide reductase), regulation of chloroplasts, as well as the replication and assembly of certain phages (3).

Glutathione reductase has been characterized to high resolution by x-ray crystallography (4) and is one of a family of related oxidoreductases that act on a variety of substrates (e.g. lipoamide dehydrogenase, trypanothione and mercuric reductases) (3). The Escherichia coli gene encoding thioredoxin reductase has been cloned (5) and its DNA sequence recently determined (6). These results show that thioredoxin reductase (34.5 kDa) is smaller than the other reductases (~50 kDa) and differs from that of glutathione reductase, and as a step toward its elucidation we report here the crystallization of the E. coli enzyme.

**MATERIALS AND METHODS**

A pure sample of the protein was prepared as described by Russel and Model (7), and was dialyzed against 10 mM HEPES' buffer (pH 7.0) and 5 mM EDTA, over a period of 12-20 h. The solution was then concentrated down to 10 mg/ml using ultrafiltration. Crystals were first obtained at room temperature, by vapor diffusion against a reservoir containing 40% polyethylene glycol (Sigma; average molecular mass of 3.3 kDa) and 200 mM ammonium sulfate. The initial hanging drop contained 1 part protein solution, 1 part reservoir solution, and 1 part 60 mM dithiothreitil. Yellow single crystals appeared within 1-2 days and grew as long hexagonal needles or rods. Crystallization experiments were also carried out at 4 °C but were not successful. For example, one tray of vapor diffusion experiments that had been stored at 4 °C for a few weeks, with only one or two crystals resulting, yielded a large number of single crystals within 2 days of moving the tray to room temperature. The reservoir solution can be made up with or without buffer, although the optimal conditions appear to be with 50 mM MES or HEPES buffer (pH 6.5-7.0), polyethylene glycol concentrations around 35%, and with protein concentrations around 20 mg/ml. Crystals also grow if 200 mM lithium sulfate is used instead of the ammonium sulfate.

**RESULTS AND DISCUSSION**

The protein solution from which the crystals were initially grown had been stored at 4 °C for approximately 2 years prior to the final dialysis. When similar crystallization conditions were set up using freshly prepared protein, no crystals were observed to grow over a period of several weeks. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5) showed no differences between the old protein solution and fresh samples. However, large single crystals were reproducibly obtained from the fresh protein solutions by "micro-seeding": two or three crystals (0.5-1.0 mm long) from a previous batch were crushed in 0.1 ml of the reservoir solution until particles were no longer visible at a magnification of × 80. The solution was then diluted by a factor of 3 and was used as the seeding solution. Vapor diffusion experiments were set up as before, except that the drop now contained 1 part seeding solution in addition to the other components. Single crystals can be observed in most drops within 4 h of seeding, and they grow to a reasonable size (0.2 × 0.2 × 1.0 mm) in about a week (Fig. 1). The crystals are of the same hexagonal morphology as those obtained without seeding from the old protein solution. We have not carried out a search for new crystallization conditions using fresh protein solution because the micro-seeding technique is reproducible.

Precipitation photographs reveal that the hexagonal morphology of the crystals is reflected in the diffraction pattern. The hkl zone (Fig. 2) shows 6mm symmetry, as does the hk0 zone, with the 6-fold axis coincident with the long axis of the crystals. The h0l and 0kl zones show mm symmetry. Precipitation photographs, as well as diffractometer data set to 4 Å

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Single crystals of thioredoxin reductase, suitable for x-ray diffraction studies, have been obtained at room temperature by vapor diffusion of 10 mg/ml protein solution against 35% polyethylene glycol containing 200 mM ammonium sulfate. Good quality crystals appear spontaneously only from a protein solution that had been stored for more than a year at 4 °C, although large single crystals are reproducibly obtained from fresh protein solutions by micro-seeding. The space group is P6_{2}2_{1} (a = b = 123.8 Å, c = 81.6 Å), with one monomer of the enzyme (34.5 kDa) in the crystallographic asymmetric unit. The crystals are well ordered and diffract to beyond 2 Å resolution.

The sequence around the active site disulfide is also distinct, with 2 residues intervening between the cystines, as opposed to 4 in the others (6). The three-dimensional structure of thioredoxin reductase is therefore expected to be quite different from that of glutathione reductase, and as a step toward its elucidation we report here the crystallization of the E. coli enzyme.
resolution, show systematic absences along 00l, with reflections absent for \( l = 2n + 1 \). This results in the assignment of the space group as \( \text{P6}_{3}22 \). The unit cell dimensions, based on diffractometer measurement of 22 strong reflections to 4 Å resolution, are \( a = b = 123.84 \pm 0.07 \) Å, \( c = 81.56 \pm 0.06 \) Å, with a unit cell volume of 1,083,229 Å\(^3\). Assuming one molecule/asymmetric unit we obtain a reasonable value of 2.6 Å\(^3\)/dalton for \( V_{m} \), the crystal packing parameter (9). The symmetry of the packing then demands that the protein form dimers in the lattice, which is consistent with the fact that the enzyme is active as a dimer (2).

Still photographs taken using a rotating-anode generator, as well as oscillation photographs taken at the Brookhaven synchrotron, show diffraction extending to 2 Å resolution. The crystals are stable in the rotating-anode x-ray beam for 2–3 days. We are currently measuring intensity data on a diffractometer and also screening for heavy-atom derivatives.

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