Crystal Structure Analysis of a Mutant *Escherichia coli* Thioredoxin in Which Lysine 36 Is Replaced by Glutamic Acid[†,‡]

Matti Nikkola,[*,§] Florence K. Gleason,[∥] James A. Fuchs,[⊥] and Hans Eklund[§]

Department of Molecular Biology, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, Uppsala, Sweden, and Departments of Plant Biology and Biochemistry, University of Minnesota, St. Paul, Minnesota

Received May 14, 1992; Revised Manuscript Received July 30, 1992

ABSTRACT: The structure of a mutant *Escherichia coli* thioredoxin with a glutamic acid substituted for a conserved lysine at position 36 adjacent to the active site has been solved using molecular replacement and refined at 2.0-Å resolution to a crystallographic residual of 19.9%. The mutant was crystallized in an orthorhombic space group with one molecule in the asymmetric unit. The structure of the mutant thioredoxin shows overall good agreement with the wild-type *E. coli* thioredoxin. The root-mean-square deviations for all Cαs are 0.45 and 0.79 Å between the mutant structure and the two molecules in the asymmetric unit of the wild-type crystals. Structural changes are seen in several residues in the active-site region preceding the disulfide. A reverse turn of residues 29–32 changes the conformation from a type I to a type II turn. This change may be related to the loss of a hydrogen bond from Lys-36 to the main-chain carbonyl of residue 30 due to the mutation. The Cα atom of Trp-31 has moved 1.9 Å and the indole ring no longer makes hydrogen bonds to the carboxyl group of Asp-61 but instead participates in a crystal contact. The structural differences seen in the mutant thioredoxin may be influenced by the crystal packing. The substituted Glu-36 makes extensive crystal contacts. The static fluorescence of this mutant thioredoxin has a different pH dependence than the wild type.

Thioredoxins are small, ubiquitous redox proteins that have been isolated from a large number of procaryotic and eucaryotic organisms (Holmgren, 1985, 1989). Thioredoxin from *Escherichia coli* was first described as a small (M_r 11 700), stable redox protein which serves as a reducing agent for ribonucleotide reductase, an enzyme essential for the biosynthesis of DNA (Laurent et al., 1964). Later, it became evident that the role thioredoxin plays in the production of deoxyribonucleotides may not be its major function. Thioredoxin from *E. coli* has been shown to be essential for the assembly of the filamentous phages M13 and f1 (Russel & Model, 1985; Lim et al., 1985). It also functions as a subunit of the DNA polymerase of phage T7 (Mark & Richardson, 1976). Thioredoxin can regulate the activity of enzymes by reducing their disulfides (Scheibe, 1991). The various functions of thioredoxin and proteins similar to thioredoxin in procaryotic organisms have been reviewed by Gleason and Holmgren (1988).

The primary structures of many thioredoxins are known. Thioredoxins vary in length from 105 to 110 amino acids, and different thioredoxins have 27–69% sequence identity to *E. coli* thioredoxin (Eklund et al., 1991). The active-site sequence Trp-Cys-Gly-Pro-Cys is conserved among all thioredoxins, and there usually is a lysine or an arginine after the second cysteine (Eklund et al., 1991).

The crystal structure of oxidized *E. coli* thioredoxin has been solved (Holmgren et al., 1975) and the structure refined at 1.68-Å resolution (Katti et al., 1990). NMR[1] structures have been reported for human and *E. coli* thioredoxins in their reduced forms (Forman-Kay et al., 1991; Dyson et al., 1990). The molecule consists of a central pleated β-sheet with both parallel and antiparallel strands, surrounded by α-helices. Because thioredoxin has a large content of secondary structure, it is a very stable protein (Kelley & Stellwagen, 1984). The redox-active disulfide is located in a protrusion in the protein at the amino end of the α2 helix (see Figure 1). Adjacent to the disulfide there is a flat hydrophobic surface, which has been thought to be important in the interaction of thioredoxin with other proteins (Eklund et al., 1984).

Thioredoxin is a protein disulfide oxidoreductase (Holmgren, 1979). The two cysteines in thioredoxin were found to have different reactivities. The more accessible Cys-32 has a low pK_a value of 6.7. It was proposed that the thiolate anion of Cys-32 was stabilized by the positively charged residue at position 36 (Kallis & Holmgren, 1980). However, the high-resolution crystal structure revealed that the side chain of the lysine was too far from the cysteine to serve this function (Katti et al., 1990). Since the structure of α-helices creates a positive charge at their N-termini (Hol et al., 1978), it was postulated that the observed low pK_a of Cys-32 was induced by the α2 helix dipole moment (Katti et al., 1990).

To resolve this issue, a mutant thioredoxin, generated by random mutagenesis that has Lys-36 substituted with a negatively charged glutamic acid, was characterized. The mutant thioredoxin remains active with thioredoxin reductase and ribonucleotide reductase at physiological pH and supports the growth of the filamentous phages M13 and T7 (Gleason et al., 1990). Kinetics show that the K_m is about 3 times higher in the reaction with thioredoxin reductase while the kinetic parameters for reduction of ribonucleotide reductase

† This study was supported by grants from the Finnish Academy, the Swedish Natural Science Research Council, the Swedish National Board for Technical Developments, the Swedish Ministry of Agriculture, and U.S. National Science Foundation.

‡ The coordinates for the K36E mutant thioredoxin have been deposited in the Brookhaven Protein Data Bank under file name 2TIR.

* Correspondence should be addressed to this author.

§ Swedish University of Agricultural Sciences.

∥ Department of Plant Biology, University of Minnesota.

⊥ Department of Biochemistry, University of Minnesota.

[1] Abbreviations: NMR, nuclear magnetic resonance; *B*-value, crystallographic temperature factor; rms, root mean square; wt, wild type; F_o, observed structure factor amplitude; F_c, calculated structure factor amplitude; *R*-factor, conventional crystallographic residual.

0006-2960/93/0432-5093$04.00/0

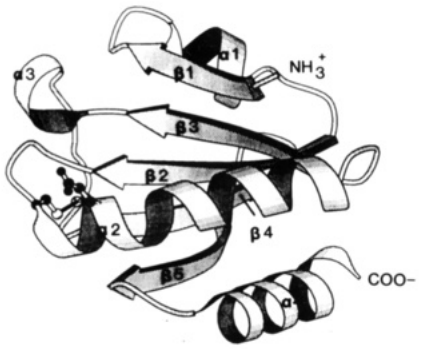

FIGURE 1: Ribbon model of the mutant *E. coli* thioredoxin K36E with the side chain of Glu-36 shown along with the side chains of the disulfide-forming cysteines 32 and 35. The secondary structure elements have been named according to Katti et al. (1990). The picture was drawn using Molscript (Kraulis, 1991).

are close to those for the wild type. Studies with stopped-flow kinetics show that a negative charge at position 36 slows binding to thioredoxin reductase by 2 orders of magnitude (Navarro et al., 1991).

In this paper we report the crystal structure of the mutant *E. coli* thioredoxin K36E. The crystal structure in relation to the biochemical properties of the mutant is discussed. The crystallographic work on thioredoxins has so far been performed on the same crystal form, which contains two molecules in the asymmetric unit. The details of these two molecules differ for parts of the molecule, especially for about 20 N-terminal residues (Katti et al., 1990). Since the mutant protein crystallizes in a different space group than the wild-type protein, the crystallographic investigation of this variant should provide an opportunity to examine the influence of crystal packing.

EXPERIMENTAL PROCEDURES

Protein Purification. Thioredoxin K36E was obtained by a random mutagenesis procedure as described by Myers et al. (1985). The mutated thioredoxin gene was ligated into pUC19 in a direction opposite the *lac* promoter. Expression of a wild-type thioredoxin gene under these conditions was sufficient to allow growth of a *metE* strain on methionine sulfoxide. Strains exhibiting reduced or no growth were identified and the mutant gene was sequenced. Isolation and characterization of this protein has been reported (Gleason et al., 1990). The protein was homogeneous as judged by silver staining of native and denaturing polyacrylamide electrophoresis gels.

Crystallization, Space Group Determination, and Data Collection. Mutant thioredoxin K36E was crystallized in 15-μL hanging drops using the vapor diffusion method in 0.015 mM sodium acetate, pH 4.8, 17% PEG 6000, and 1 mM cupric acetate at 4 °C. Large, single crystals of the mutant protein in the shape of hollow hexagonal pipes appeared in 2–3 weeks. The crystals were very thin-walled and typically 0.3 mm wide and 1–5 mm long. Long, thin crystals of thioredoxin K36E were formed without the addition of cupric acetate to the hanging droplets. However, the presence of millimolar concentrations of cupric ions was found to improve the quality of the crystals greatly, and all of the diffraction data was collected with crystals formed in drops containing cupric ions.

The space group was determined from zero-level precession photographs and diffraction data collected on three protein crystals on a Xentronics (Nicolet) area detector mounted on a Rigaku rotating anode at 9 °C. The crystals were stable in the beam and they diffracted beyond 2.0-Å resolution. The crystals belong to the space group $P2_12_12_1$ with unit cell dimensions 26.8, 50.8, and 80.7 Å and one protein molecule per asymmetric unit. There is 46% solvent in the crystals.

The data were processed with the BUDDHA program package (Blum et al., 1986). The crystal data sets were scaled with the PROTEIN program package (Steigemann, 1974). A total of 31 697 measurements merged to 7477 unique reflections with an R-value of 8.7%. Some of the raw data (4.1%) were discarded in the scaling process. The final data set used in the refinement has a merging R-value of 7.57‰ for intensities and contains 73% of all data from 7 to 2 Å.

Molecular Replacement. The whole molecule A in the refined 1.68-Å structure of oxidized *E. coli* thioredoxin (Katti et al., 1990) with no modifications was used as the search model. The rotational parameters of the molecular replacement protein model in the orthorhombic unit cell were determined using the program POLARRFN, by Wolfgang Kabsch (1984) (included in the CCP4 program package), and refined by doing a search around the highest peak using a fine grid of 1°. The structure factors for the model were calculated in a triclinic cell with orthogonal axes of 60 Å. The rotation function indicated a single peak. The clearest result was achieved using an integration radius of 15 Å and a data resolution range of 8–2 Å.

The translation correlation function of the program package X-PLOR (Brünger et al., 1987) was used to determine the position of the correctly rotated search model in the orthorhombic unit cell. The translation function at 15–4-Å resolution showed a single peak. The results of the rotation and translation functions were unambiguous and the whole molecular replacement procedure was completed in 2 days.

Refinement. The rotated and translated search model was subjected to rigid-body refinement in X-PLOR. After rigid-body refinement the R-factor was 43% for reflections from 20- to 5-Å resolution. The correctly positioned model was then refined with simulated annealing at high temperatures (X-PLOR) alternated with building sessions at a molecular graphics display using the graphics program O (Jones et al., 1990). Isotropic B-factors were refined for all atoms. The present R-factor for all data between 7.0 and 2.0 Å is 19.9%. A total of 56 water molecules were added gradually during the refinement at peaks found in the $|F_o|-|F_c|$ difference maps.

The N-terminus has a structure that is different from the structures of the N-termini of the two wild-type molecules A and B. Inspection of difference maps and omit maps calculated without the first few residues made it possible to locate these residues and the cupric ion that binds to the N-terminus.

The backbone torsion angles ϕ and ψ (Ramachandran et al., 1963) for the mutant thioredoxin are shown in Figure 2. Arg-73 makes contact with Trp-31 and Ile-75 in a crystallographically related molecule. It has a different conformation from that found in the wild-type structure and the backbone torsion angles are slightly outside the allowed region (Figure 2).

The coordinates have been deposited in the Brookhaven Protein Data Bank under file name 2TIR.

Fluorescence Spectra. Fluorescence spectra were obtained using a Perkin-Elmer MPF 44A spectrometer. Measurements were made in a 1-cm path length cell in a total volume of 1.0 mL. The excitation wavelength was 280 nm and emission was recorded from 290 to 400 nm at 30 °C. Spectra were compared to those of *E. coli* wild-type thioredoxin produced under similar conditions. Thioredoxins were dissolved in the following buffers (100 mM, containing 1 mM EDTA) for determination of pH-dependent fluorescence emission: NaAc,

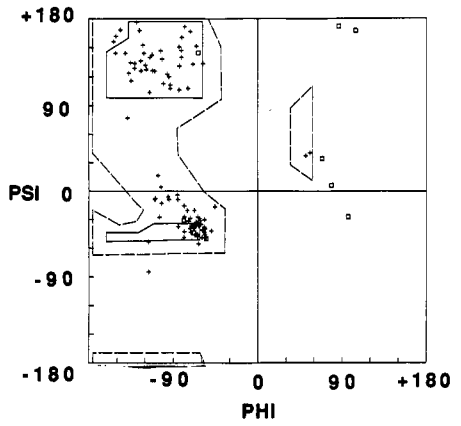

FIGURE 2: Ramachandran plot of thioredoxin K36E. Squares indicate glycines and crosses non-glycines.

pH 5.0; KPO_4, pH 5.5–7.5; Tris-HCl, pH 8.0–9.0; ethanolamine hydrochloride, pH 9.5–10.5. Quantum yields were calculated from the area of the spectra as compared to that of tryptophan in H_2O. Spectra were not corrected for detector responses.

Denaturation of Thioredoxin with Guanidine Hydrochloride. The relative stabilities of oxidized wild-type thioredoxin and thioredoxin K36E were measured by equilibrium denaturation studies with guanidine hydrochloride. Thioredoxins were dissolved in different concentrations of guanidine hydrochloride prepared in 100 mM potassium phosphate buffer and adjusted to pH 7.0. The midpoints of the denaturation curves were estimated by plotting the concentration of guanidine hydrochloride versus ΔG_D, where $\Delta G_D = -RT \ln K_{eq}$. The $\Delta\Delta G_D$ was estimated by multiplying the denaturation midpoint by the slope of the line as described in Pace (1986).

RESULTS

The structure of the mutant thioredoxin K36E was solved by molecular replacement and has been refined to an *R*-factor of 19.9% at 2.0-Å resolution. The refined model has good stereochemistry with a root-mean-square deviation for bond lengths of 0.017 Å and for bond angles of 2.95°.

The electron density map is well-defined for most of the molecule, including the active-site residues. The electron density map ($2|F_o|-|F_c|$) around the side chain of the substituted glutamic acid at position 36 is shown in Figure 3. The side chain of Glu-36 makes a crystal contact to a symmetry-related molecule. Likewise, the side chain of the active-site residue Trp-31 makes a crystal contact and has strong, well-defined density (Figure 4).

Comparison to Wild-Type Thioredoxin. The mutant thioredoxin and the two wild-type molecules have similar conformations. The β-sheet is practically identical in the molecules. For Cα atoms the rms difference from wild-type molecule A is 0.45 Å, and from the less well-defined wild-type molecule B, 0.79 Å. The largest differences are found for residues 1–4 and 28–36 around Trp-31. The main chain also differs for residues in a few bends at the surface (Figure 5). The α1 helix of thioredoxin K36E has the more ordered structure of the corresponding helix in the wild-type molecule A.

The *B*-factors of the thioredoxin K36E backbone (averaged for C, C_α, N, and O) follow the overall pattern of the two molecules of the wild-type thioredoxin crystal structure (Nikkola, 1991). The greatest differences are seen for α1, where wild-type molecule B is disordered.

Structure of the Active Site. The largest structural differences of the K36E mutant compared to wild-type thioredoxin are seen in the active site. Residues 29–32 form a reverse turn of type I in the wild-type molecules with a hydrogen bond from 29 O to N 32. The carbonyl oxygen of Cys-32 then forms the first hydrogen bond of α2 to N 36. The reverse turn may be stabilized by a hydrogen bond to the main-chain carbonyl of residue 30 in the bend to the side chain of Lys-36. In the K36E mutant this hydrogen bond cannot be formed and the peptide has flipped its direction by about 180°. The reverse turn is thus changed from a type I turn to a distorted type II turn (Richardson, 1981). The main-chain torsion angles of residues 30 and 31 are (–72°, 167°) and (51°, 37°), respectively.

The conformation of the indole ring of Trp-31 is also very different from that found in the wild-type structure. The residues around the disulfide, including Trp-31, superimposed on the corresponding residues from the wild-type molecule A are shown in Figure 6. In both wild-type molecules the NE1 of Trp-31 forms hydrogen bonds to the carboxyl oxygen atoms of Asp-61 (Katti et al., 1990). This bond is not present in the mutant structure. The side-chain torsion angles χ_1 and χ_2 change from 41° and 95° in the wild-type protein to –49° and –62° in the K36E mutant.

The side chain of Glu-30 also changes its position in the transition of the reverse turn. In the wild type it is 3.4 Å from Trp-28, but in the K36E mutant protein it has moved away from Trp-28 and is closer to Trp-31. By this movement it has moved away from residue 36 by about 4 Å.

The disulfide bridge has normal dihedral angles similar to those found in wild-type thioredoxin (Katti et al., 1990). The dihedral angles for Cys-32 are $\chi_1 = 176°$ and $\chi_2 = -140$ °C. Cys-35 has dihedral angles $\chi_1 = 81°$ and $\chi_2 = -59°$. The torsion angle about the disulfide bond is 69°. The S–S bond is 2.04 Å and the Cαs of the cysteines are 5.2 Å apart. The two sulfur atoms have shifted their positions slightly from the positions they take in the wild-type structure.

Much of the active site is involved in direct or water-mediated crystal contacts. The carboxyl oxygen atoms OE1 and OE2 of Glu-36 make intermolecular hydrogen bonds to the OG1 and the backbone N of Thr 89. The side-chain nitrogen atom of Trp-31 forms a hydrogen bond to the main-chain carbonyl of residue 70 in another molecule. Trp-31 also makes extensive van der Waals contacts with Arg-73 and Gly-74 from a third molecule.

Solvent Structure and Cu Binding. The solvent structure contains 56 water molecules, including the one making hydrogen bonds to Lys-57 and Asp-26. Many of the water molecules take similar positions as waters found in either molecule of the wild-type structure.

Copper binding is different from the binding in native thioredoxin crystals but involves the same residues, Asp-2 (both side-chain carboxyl and main-chain nitrogen) and Asp-10 from another molecule.

Fluorescence Studies. The effect of pH on fluorescence of oxidized and reduced thioredoxin K36E is shown in Figure 7. The fluorescence intensity for reduced thioredoxin K36E has a maximum at pH 6 and decreases at higher pH values. The intensities for the oxidized form are lower at all pH values measured and do not vary with pH.

Stability Studies. The guanidine hydrochloride denaturation curves of wild-type thioredoxin and thioredoxin K36E are shown in Figure 8. The midpoint of denaturation has shifted slightly from 3.4 M for wild-type thioredoxin to 3.0

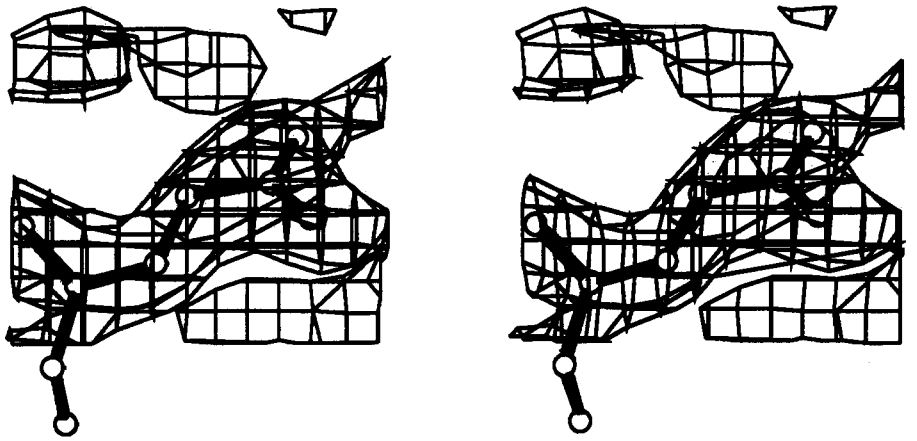

FIGURE 3: $2|F_o| - |Fc|$ electron density map of the Glu-36 side chain. The map has been contoured at 1σ level.

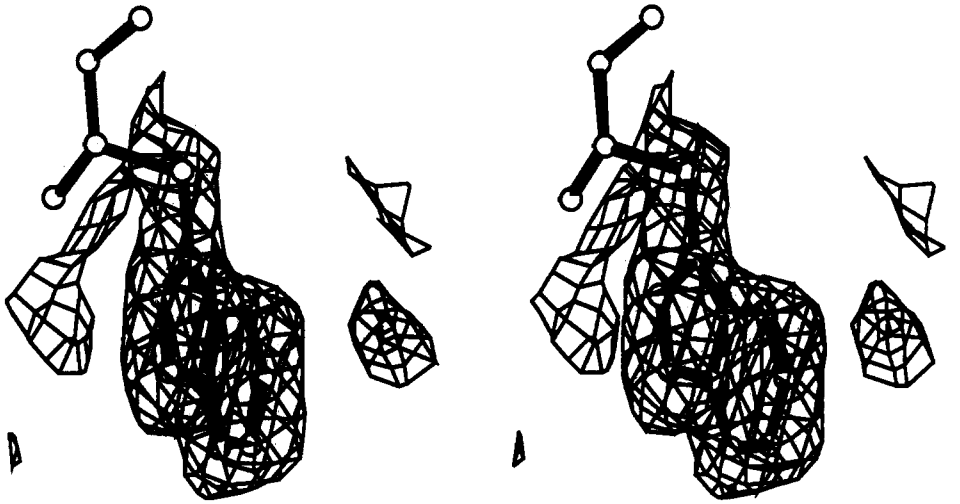

FIGURE 4: $2|F_o| - |F_c|$ electron density map of the side chain of Trp-31. The map has been contoured at 1σ level.

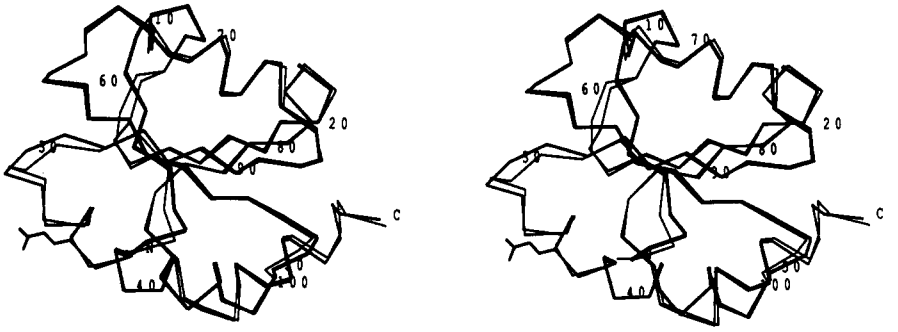

FIGURE 5: Stereoview of the Cα backbones of the A molecule of the wild-type structure and thioredoxin K36E superposed with a least-squares method in O (Jones et al., 1990).

M for the mutant thioredoxin. The mutant protein is less stable than the wild type by 0.36 kcal/mol.

DISCUSSION

Crystallization and the Packing of Crystals. The crystallographic investigations of *E. coli* thioredoxin have previously been performed on monoclinic crystals formed in the presence of Cu^{2+} ions and with two molecules in the asymmetric unit (Holmgren et al., 1975; Katti et al., 1990). The refined structure of thioredoxin shows that the active-site region is very similar in the two wild-type molecules and that the differences increase with the distance from the active site (Katti et al., 1990). The largest differences were found for helix α1, which is disordered in molecule B. This is in contrast, to the K36E thioredoxin, where the differences are found in the active site.

The crystallization procedure for thioredoxin K36E differs from that used for wild-type *E. coli* thioredoxin (Holmgren & Söderberg, 1973; Katti et al., 1990). PEG [poly(ethylene glycol), MW 6000] and the hanging drop method were used to crystallize thioredoxin K36E. An active-site insertion mutant of thioredoxin has been crystallized in the hexagonal space group $P6_1$ using the same procedure. Several other mutant thioredoxins have been crystallized and they all crystallize in space groups other than the original form for wild-type protein (at least four different space groups so far) except for thioredoxin P34S (M.N., unpublished results). All of them have been crystallized in hanging drops using NaAc, pH 3.8–5.0, CuAc, and PEG 6000. This tendency to change forms is different from that of T4 lysozyme, for which about 80% of the many mutants investigated crystallize in the same space group.

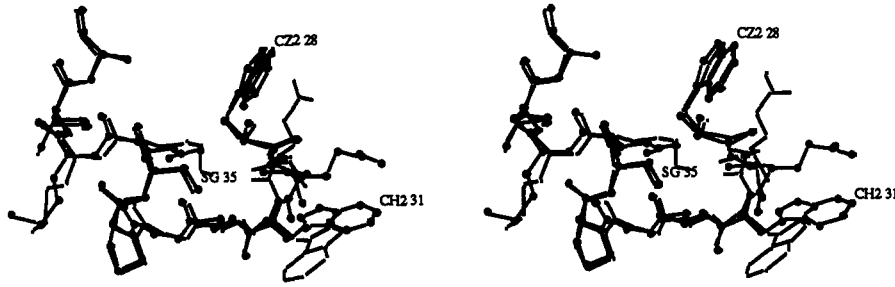

FIGURE 6: Active-site residues 28–39 of thioredoxin K36E superposed with those of wild-type thioredoxin, molecule A. The mutant structure is indicated by spheres.

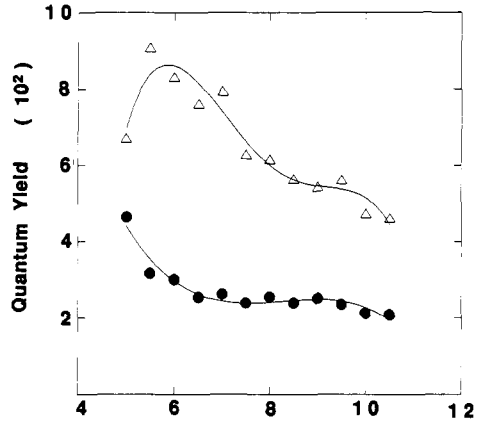

FIGURE 7: Effect of pH on fluorescence of thioredoxin K36E. Thioredoxin was dissolved in buffers at different pH's as described under Experimental Procedures. After the spectrum of the oxidized form was obtained, the sample was reduced with 1 mM (final concentration) dithiothreitol. The excitation wavelength was 280 nm. Quantum yields were calculated on the basis of a Q of tryptophan in water equal to 0.144 (Wiget & Luisi, 1978). The quantum yields are plotted vs pH for oxidized thioredoxin K36E (●) and reduced (△).

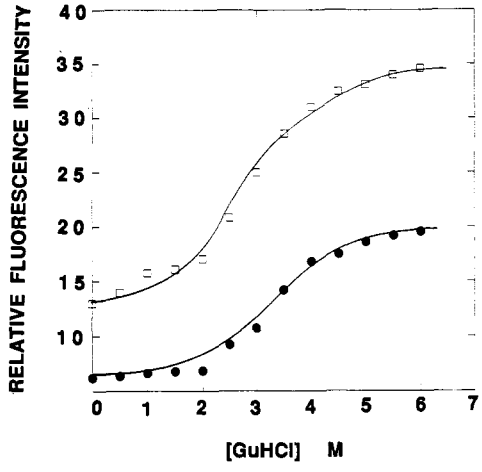

FIGURE 8: Guanidine hydrochloride denaturation curve of oxidized thioredoxins. Samples were prepared by adding thioredoxins to various concentrations of guanidine hydrochloride at pH 7.0 as described under Experimental Procedures. Relative fluorescence intensity is plotted versus guanidine hydrochloride concentration for wild-type thioredoxin (●) and thioredoxin K36E (□).

Many of the differences in the structure of the mutant thioredoxin compared to the wild-type molecules may be due to packing rather than changes in protein structure due to the mutation. The new glutamic side chain interacts with atoms in another molecule that are not present in the wild-type crystals. In addition, new intermolecular interactions by Trp-31 and Arg-73 are formed.

Structural Changes at the Active Site. The most striking difference in the three-dimensional structure of the K36E mutant protein compared to the wild-type molecules is that the reverse turn of residues 29–32 has changed its conformation. This may be due to the loss of the hydrogen bond formed in the wild-type molecules between the side chain of Lys-36 and the carbonyl oxygen of residue 30. A Glu at position 36 cannot form this bond. Surprisingly, this results in a new conformation of the side chain of Trp-31. This residue is conserved in all thioredoxins and the arrangement of this side chain seems important. The residues that maintain its conformation, Ala-29, which is buried behind the indole ring, and Asp-61, which forms hydrogen bonds to NE1, are also highly conserved (Eklund et al., 1991). The position of Trp-31 in reduced thioredoxin is well determined by 2D-NMR and there is very little difference between the different calculated molecules (Dyson et al., 1990). The ring of Trp-31 also superimposes very closely in the NMR and X-ray structures. However, the very different conformation of this side chain in the K36E mutant structure may be partly due to crystal packing.

Since both Trp-28 and Trp-31 are in the region of the active-site disulfide, changes in static fluorescence of thioredoxin are a sensitive indicator of the conformation and redox state. The fluorescence behavior of the thioredoxin K36E shows subtle changes from that of wild type. As previously reported, the mutant protein exhibits a 2-fold increase in quantum yield on reduction compared to a 3-fold increase for the wild-type thioredoxin (Gleason et al., 1990). This increase, mainly due to loss of disulfide quenching around Trp 28, was shown to be pH dependent (Holmgren, 1972). However, in thioredoxin K36E, the fluorescence maximum occurs at pH 6.0, versus pH 5.0 in the wild type, and the titrating group exhibits a slightly higher pK_a. These changes indicate that the environment of the tryptophans in the mutant protein is altered. Thus the shifts seen in the crystal structure in the positions of residues in the active site indicate that the mutation has also altered the protein conformation in solution. The observed change in stability could be the effect of local distortion caused by electrostatic repulsion of Glu 30 and Glu 36 and the aforementioned loss of a hydrogen bond.

Role of Lys-36. Thioredoxin K36E was originally selected as a mutant with decreased ability to reduce methionine sulfoxide to methionine following saturation mutagenesis of the *trxA* gene on a plasmid (Russel & Model, 1986). The K36E mutation causes only minor changes in the viability of *E. coli* but was studied in detail since it had been proposed that the thiolate anion of Cys-32 was stabilized by the positively charged residue at position 36 (Kallis & Holmgren, 1980). The observation indicates that this is a powerful method of obtaining *E. coli* thioredoxin mutants since it allows selection of proteins with only minor alterations.

Thioredoxin interacts with a number of different proteins such as thioredoxin reductase and ribonucleotide reductase. The specificity of these interactions is due to the conformation

or thioredoxin and the different functional groups of the residues found on its surface. The crystal structure of thioredoxin K36E shows that the glutamic acid residue in the mutant protein occupies a similar position on the surface near the disulfide as does lysine in the wild-type. Since both the X-ray and fluorescence data indicate that the mutation causes small overall effects on structure and stability, changes in activity must be due mainly to substitution of the opposite charge near the active site. The conserved positive charge at position 36 is not absolutely essential to thioredoxin activity since the mutant protein can still function both as a substrate for thioredoxin reductase and as a reducing agent with approximately 75% of the catalytic efficiency of the wild-type protein (Gleason et al., 1990). It can be concluded that the pK_a of cysteine 32 is lowered by its interaction with the $\alpha 2$ helix dipole. The positively charged residue is conserved at position 36 to optimize protein interactions and it does not increase the reactivity of the active-site thiol group.

REFERENCES

Blum, M., Metcalf, P., Harrison, S. C., & Wiley, D. C. (1987) *J. Appl. Crystallogr. 20*, 235–242.

Brünger, A. T., Kuriyan, J., & Karplus, M. (1987) *Science 235*, 458–460.

Dyson, H. J., Gippert, G. P., Case, D. A., Holmgren, A., & Wright, P. E. (1990) *Biochemistry 29*, 4129–4136.

Eklund, H., Cambillau, C., Sjöberg, B.-M., Holmgren, A., Jörnvall, H., Höög, J.-O., & Brändén, C.-I. (1984) *EMBO J. 3*, 1443–1449.

Eklund, H., Gleason, F. K., & Holmgren, A. (1991) *Proteins: Struct., Funct., Genet. 11*, 13–28.

Forman-Kay, J. D., Clore, G. M., Wingfield, P. T., & Gronenborg, A. M. (1991) *Biochemistry 30*, 2685–2698.

Gleason, F. K., & Holmgren, A. (1988) *FEMS Microbiol. Rev. 54*, 271–298.

Gleason, F. K., Lim, C.-J., Gerami-Nejad, M., & Fuchs, J. A. (1990) *Biochemistry 29*, 3701–3709.

Hol, W. G. J., van Duijnen, P. T., & Berendsen, H. J. C. (1978) *Nature 273*, 443–446.

Holmgren, A. (1972) *J. Biol. Chem. 247*, 1992–1998.

Holmgren, A. (1979) *J. Biol. Chem. 254*, 9113-9119.

Holmgren, A. (1985) *Annu. Rev. Biochem. 54*, 237–271.

Holmgren, A. (1989) *J. Biol. Chem. 264*, 13963–13966.

Holmgren, A., & Söderberg, B.-O. (1970) *J. Mol. Biol. 54*, 387–390.

Holmgren, A., Söderberg, B.-O., Eklund, H., & Brändén, C.-I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2305–2309.

Jones, T. A., Bergdol, M., & Kjeldgaard, M. (1990) in *Molecular modelling* (S. Ealick, S., & Bugg, C. A., Eds.) pp 189–199, Springer, New York.

Kabsch, W. (1984) in CCP4 Program Suite, Rotation Function POLARRFN, User Documentation, Daresbury Laboratory, Daresbury, U.K.

Kallis, G.-B., & Holmgren, A. (1980) *J. Biol. Chem. 255*, 10216-10261.

Katti, S. K., LeMaster, D. M., & Eklund, H. (1990) *J. Mol. Biol. 212*, 167–184.

Kelley, R., & Stellwagen, E. (1984) *Biochemistry 23*, 5095–5102.

Kraulis, P. J. (1991) *J. Appl. Crystallogr. 24*, 946–950.

Laurent, T. C., Moore, E. C., & Reichard, P. (1964) *J. Biol. Chem. 239*, 3436–3444.

Lim, C.-J., Haller, B., & Fuchs, J. A. (1985) *J. Bacteriol. 161*, 799–802.

Mark, D. F., & Richardson, C. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 780–784.

Myers, R. M., Terman, L. S., & Maniatis, T. (1985) *Science 229*, 242–247.

Navarro, J. A., Gleason, F. K., Cusanovich, M. A., Fuchs, J. A., Meyer, T. E., & Tollin, G. (1991) *Biochemistry 30*, 2192–2195.

Nikkola, M. J. (1991) Ph.D. Thesis, Swedish Agricultural University, Uppsala, Sweden.

Pace, N. (1986) *Methods Enzymol. 131*, 266–280.

Ramachandran, G. N., Ramakrishnan, C., & Sasisekharan, V. (1963) *J. Mol. Biol. 7*, 95–99.

Richardson, J. (1981) *Adv. Protein Chem. 34*, 168–339.

Russel, M., & Model, P. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 29–33.

Russel, M., & Model, P. (1986) *J. Biol. Chem. 261*, 14997–15005.

Scheibe, R. (1991) *Plant. Physiol. 96*, 1–3.

Steigemann, W. (1974) Ph.D. Thesis, Technische Universität Munich, Germany.

Wiget, P., & Luisi, P. L. (1978) *Biopolymers 17*, 167–180.