Biochemistry

 Volume 34, Number 32 August 15, 1995

Accelerated Publications

Proton Sharing between Cysteine Thiols in *Escherichia coli* Thioredoxin: Implications for the Mechanism of Protein Disulfide Reduction†

Mei-Fen Jeng,‡ Arne Holmgren,§ and H. Jane Dyson*,‡

The Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037, and Medical Nobel Institute for Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-17177 Stockholm, Sweden

Received May 10, 1995⊗

ABSTRACT: Proton sharing between acidic groups has been observed in the active sites of several enzymes, including bacteriorhodopsin, aspartic proteases, and ribonuclease HI. We here report NMR observations suggestive of proton sharing between cysteine thiols in the active site of the oxidation–reduction enzyme thioredoxin. The pK_as of the two cysteine thiols in the *Escherichia coli* protein are removed from the expected value of 8.4 by ~1 pH unit in either direction, upward and downward. Further, the C^β resonances of both residues show clearly the effects of both of these pK_as, indicating that the titrations of the two thiol groups are intimately linked. This behavior strongly suggests that the low pK_a ascribed to the deprotonation of the Cys 32 thiol most likely arises through the interaction and close approach of the thiol of Cys 35, with the thiolate anion of Cys 32 stabilized through the sharing of the remaining thiol proton, nominally attached to Cys 35. These observations provide a rationale for the mediation of active site pH control, an important aspect of the mechanism of thioredoxin and other proteins with catalytic thioredoxin domains, such as protein disulfide isomerases.

Thioredoxins are small (M_r ~12 000), ubiquitous proteins that mediate oxidation–reduction reactions in a variety of cellular and virus reactions (Holmgren, 1985) and are homologous structurally and functionally to mammalian protein disulfide isomerase (Krause *et al.*, 1991) and the *Escherichia coli* disulfide isomerase DsbA (Martin *et al.*, 1993). The best characterized thioredoxin is the protein from *E. coli*, which contains 108 amino acids, with the active site consisting of the sequence $Trp_{31}Cys_{32}Gly_{33}Pro_{34}Cys_{35}$. Both the oxidized (Trx-S_2) and reduced [Trx-$(SH)_2$] forms of *E. coli* thioredoxin are stable under normal physiological conditions and have been characterized structurally by X-ray crystallography (Holmgren *et al.*, 1975; Katti *et al.*, 1990) and NMR (Dyson *et al.*, 1990; Jeng *et al.*, 1994). A major function of thioredoxin in living systems is to be a protein disulfide reductase, via a thiol–disulfide exchange mechanism. This reaction involves the nominal transfer of two hydrogen atoms, although under physiological conditions this is much more likely to proceed via the concerted transfer of protons and electrons. The reaction rate is therefore critically dependent on pH control in the active site region. A pK_a significantly lowered from "normal" values was observed for the Cys 32 thiol in Trx-$(SH)_2$ using iodoacetate and iodoacetamide as probes of the presence of thiolate (Kallis & Holmgren, 1980). A series of NMR studies as a function of pH (Dyson *et al.*, 1991) showed that the majority of the thioredoxin molecule is remarkably stable to pH changes

† This work was supported by Grant GM43238 from the National Institutes of Health (H.J.D.) and Grant 13x-3529 from the Swedish Medical Research Council and the P. A. Hedlunds Foundation (A.H.).

* To whom correspondence should be addressed.
‡ The Research Institute of Scripps Clinic.
§ Karolinska Institute.

⊗ Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0006-2960/95/0434-10101$09.00/0

above ~5.7 (below this pH the protein aggregates and precipitates as its p*I* is approached). In Trx-S_2 a titrating group with pK_a 7.5 was identified as the carboxyl side chain of a buried aspartate, Asp 26, near the active site, and this assignment was confirmed by mutant studies (Langsetmo *et al.*, 1991). Although the pH dependence of the NMR spectrum in the vicinity of the active site of Trx-$(SH)_2$ is complicated by the titration of three groups, the two cysteine thiols and Asp 26, the ^{1}H NMR data could be fitted to a two-proton titration with pK_as of 7.1 and 8.4 (Dyson *et al.*, 1991). In order to provide unambiguous estimates of the pK_as of these groups, we have utilized heteronuclear spectroscopy to separate and characterize the individual titrations. This paper describes the results of the titration of the two cysteine residues in a sample of reduced thioredoxin that has been semispecifically labeled with ^{13}C at the two cysteine residues.

MATERIALS AND METHODS

The *E. coli* strain JF521/pUC118trxA (Langsetmo *et al.*, 1991) was grown in 1000 mL of LB medium (10 g of Bacto tryptone, 5 g of yeast extract, and 10 g of NaCl) to which 50 mg of ampicillin and 50 mg of [$^{13}C^{\beta}$]-L-cysteine in water at pH 8.0 was added. Growth was carried out with orbital shaking at 37 °C to an OD_{650} of 1.0. The cells were induced by addition of IPTG to 0.1 mM, and growth was continued overnight. Cells were harvested by centrifugation to give 9 g wet weight. The cells were resuspended in 45 mL of 50 mM Tris-HCl−1 mM EDTA, pH 7.5, containing 1 mM PMSF and sonicated. Purification of thioredoxin then followed the protocol previously described (Dyson *et al.*, 1989) to yield 35 mg of pure protein after Sephadex G-50 chromatography.

The sample to be used for NMR was dissolved in 0.1 M potassium phosphate buffer in 99.9% ^2H_2O, pH 5.7, and reduced according to published methods (Chandrasekhar *et al.*, 1991). pH values represent meter readings uncorrected for the deuterium isotope effect.

All NMR experiments were carried out at 298 K on Bruker AMX spectrometers operating at 500 and 600 MHz for protons. The pH dependence of the $^{13}C^{\beta}$ and H^{β} resonances was determined by acquiring a series of ^{13}C−^{1}H HSQC spectra (Bax *et al.*, 1990; Norwood *et al.*, 1990) under the same conditions except for a change of pH of approximately 0.5 unit. The first measurements were made at low pH, and the pH increased by the addition of small aliquots of 0.1 M NaOD. A second series of measurements was made by repeating the above sequence, following a buffer exchange into fresh phosphate buffer at pH 5.7, to avoid changes due to differing salt strengths. ^{13}C HSQC spectra were acquired with combined States−TPPI quadrature detection, with a spectral width of 7042 Hz and 2048 complex points in ω_2 (^{1}H) and 11 000 Hz and 128 complex points in ω_1. Spectra were directly referenced to dioxane (3.75 ppm) in the ^{1}H dimension and indirectly referenced in the ^{13}C dimension.

Plots of chemical shift as a function of pH were fitted using the program Templegraph (Mihalisin Associates), using the formulas (Dyson *et al.*, 1991)

$$\delta = \delta_{HA} - \{(\delta_{HA} - \delta_A)/[1 + 10^{n(pK_a - pH)}]\}$$

for single-pK_a titrations and

$$\delta = \{1/([H^+]^2 + [H^+]K_1 + K_1K_2)\}(\delta_{H2A}[H^+]^2 + \delta_{HA}K_1[H^+] + \delta_A K_1K_2)$$

for double-pK_a titrations, where δ is the observed chemical shift at a given pH, δ_{H2A}, δ_{HA}, and δ_A are the chemical shifts for the various protonated forms of the protein, and K_a, K_1, and K_2 are acid ionization constants for the single-proton titration and for the low-pH and high-pH proton transfers, respectively, in the double-proton titration. The data points above pH 10 show larger differences than expected from the data at lower pH for a number of buried residues, including Cys 35, while the data for surface-accessible residues such as Cys 32 are relatively unaffected. Since this is probably due to the start of unfolding of the protein induced by the high pH, these points were omitted from the curve fits for the Cys 35 proton data. The last four data points for the Cys 32 $H^{\beta 2}$ proton were not included in the fit, since the $^{13}C^{\beta}-H^{\beta 2}$ cross peak was heavily overlapped at these pH values.

RESULTS

Thioredoxin enriched with ^{13}C at the C^{β} of the two cysteine residues was prepared by growing *E. coli* containing the expression plasmid in rich media to which had been added [3-^{13}C]cysteine. While this method undoubtedly results in more leakage of the label to other amino acids through transamination than would result from the careful employment of bacterial strains deficient in cysteine biosynthesis, it was found that sufficient label was incorporated unchanged into the cysteine residues of the thioredoxin to constitute a significant enrichment of label over background levels and over levels present in other amino acids through leakage. By contrast, uniform ^{13}C labeling of thioredoxin gives a product for which the cysteine $C^{\beta}-C^{\beta}H$ connectivities in the ^{13}C HSQC spectrum are difficult to see due to relatively low intensity or overlap (Chandrasekhar *et al.*, 1994). The method presented here represents a convenient compromise between the excessive and ambiguous information obtained from uniform labeling and the considerable labor involved in producing truly specifically labeled protein.

The pH titration of the thiol groups of Cys 32 and Cys 35 can be site-specifically followed by following the $C^{\beta}-H^{\beta}$ cross peaks in a ^{13}C HSQC spectrum of the cysteine-enriched sample. Especially for the C^{β}, the only influence on the observed behavior as a function of pH should be the titration of the attached −SH, enabling the titrations of the two thiols to be separated from each other and from the titration of Asp 26. The pH dependence of the HSQC spectrum is illustrated in Figure 1. The cross peaks arising from the two cysteine residues are the most intense in the spectrum and can be followed as the pH is changed. The behavior of these resonances as a function of pH is shown in Figure 2. Also shown are the data points obtained from the former ^{1}H NMR study of the pH-dependent behavior of Trx-$(SH)_2$ (Dyson *et al.*, 1991); the data from the two studies are virtually indistinguishable despite being recorded at slightly different temperatures (a slight shift to higher pH may be present for the data at the lower temperature).

It is immediately noticeable that the cysteine C^{β} titrations both show evidence of more than the single titration expected for each thiol. The pK_as obtained from fitting the data according to published methods (Dyson *et al.*, 1991) are

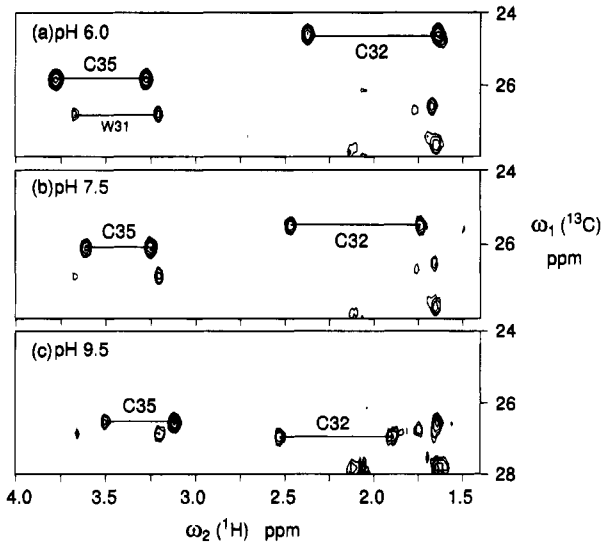

FIGURE 1: Portions of the 600 MHz $^{13}C-^{1}H$ HSQC spectra at 298 K of semi-specifically labeled Trx-$(SH)_2$, showing the behavior of the $C^{\beta}-H^{\beta}$ cross peaks of Cys 32 and Cys 35 as a function of pH: (a) pH = 6.00, contour level 0.4; (b) pH = 7.50, contour level 0.2; (c) pH = 9.46, contour level 0.1.

shown in Table 1, together with previously published estimates (Dyson *et al.*, 1991; Kallis & Holmgren, 1980). The value for the lower pK_a is comparable to those obtained previously, but the higher pK_a obtained from the Cys 32 titration curve clearly takes on a value greater than 9, closer to that obtained in the original chemical modification study (Kallis & Holmgren, 1980), rather than that obtained from previous NMR (Dyson *et al.*, 1991) and Raman (Li *et al.*, 1993) studies. We attribute the difference between the values obtained from the two NMR studies to limitations of both data acquisition and fitting in the earlier study, which incorporated ^{1}H data only, and for which only two of the three pK_as were fitted. The value obtained for the higher pK_a from the Cys 35 titration curve is even higher and not well determined by the available data. The data points above pH 10 for Cys 35 are not consistent with those at lower pHs, both for ^{1}H and for ^{13}C. This is probably due to the start of unfolding of the protein at high pH: the effect is more noticeable for the buried Cys 35 (and for other buried groups in the protein) than for the more surface-exposed Cys 32.

The two titrations are separated from each other by about 2 pH units. The normal pK_a for the cysteine thiol in a protein is 8.5 ± 0.5 (Lindley, 1960). The two cysteine pK_as are shifted from normal, in opposite directions. This is an indication of strong interactions between the two groups (Oda *et al.*, 1994), which have been interpreted as a sharing of the proton remaining on one of the groups after the ionization of the other group, by comparison with the behavior of dicarboxylic acids such as maleic acid (Pearl & Blundell, 1984).

While the two C^{β} resonances show clear indications of two pK_as, the attached protons, as noted previously (Dyson *et al.*, 1991), are apparently influenced by one or other of the pK_as, but not both. The exception to this is $H^{\beta 2}$ of Cys 32, which clearly shows two pK_as, with values similar to those seen for the C^{β} resonances. The lower of the two pK_as observed for the H^{β} resonances appears to be lower than that observed for the C^{β} resonances. Although this difference is almost within experimental error, it is a consistent trend and may reflect the influence of the Asp 26 titration on the H^{β} (but not the C^{β}) resonances, since this group is in close proximity, particularly to Cys 35, and its pK_a is about 7.4 (Dyson *et al.*, 1991; Langsetmo *et al.*, 1991). The general behavior of the H^{β} resonances as a function of pH differs for the two cysteines: the Cys 32 resonances both move downfield with increasing pH, while the Cys 35 resonances move upfield. Also, the chemical shifts of these protons are shifted from the "random" value of 2.97 ppm (Merutka *et al.*, 1995), upfield for Cys 32 and downfield for Cys 35. Thus the change in chemical shift as the pH is raised occurs for both cysteines toward the random value. The upfield-shifted H^{β} resonances of Cys 32 may reflect in part the proximity of the aromatic ring of the adjacent Trp 31, but it appears likely from the reciprocal movement of the resonances with pH that the chemical shifts themselves reflect the coupling and close association of the two cysteines.

DISCUSSION

The mechanism of reduction by thioredoxin has been proposed to proceed via a mixed-disulfide intermediate, formed by nucleophilic attack by a thiolate at Cys 32. This idea appears reasonable in view of the apparent lowered pK_a of the Cys 32 thiol (Kallis & Holmgren, 1980). However, a puzzling feature of this mechanism is the source of stabilization of the thiolate anion. The pK_a assigned to this group is more than 1 pH unit lower than the normal pK_a of a thiol in a protein, yet it is quite solvent-exposed in Trx-$(SH)_2$ (Jeng *et al.*, 1994), consistent with an important role in the initiation of reaction with protein substrates. It was originally proposed that a nearby basic residue, Lys 36, provided a positively charged group to stabilize the negative charge of the deprotonated thiol of Cys 32 (Kallis & Holmgren, 1980). However, the structure of Trx-$(SH)_2$ shows the side chain of Lys 36 to be distant from Cys 32 (Dyson *et al.*, 1990; Jeng *et al.*, 1994), and there are no other basic residues nearby. Other means of stabilization of the thiolate have been suggested, including hydrogen bonding between the Cys 32 thiol(ate) and the backbone amide proton of Cys 35 (Katti *et al.*, 1990; Forman-Kay *et al.*, 1991) and a helix−dipole effect from the long, bent helix that stretches from residues 35 to 50 (Katti *et al.*, 1990). A Cys 32 S−Cys 35 NH hydrogen bond is seen in both the X-ray and NMR structures of Trx-S_2 (Katti *et al.*, 1990; Jeng *et al.*, 1994), but it is noticeably absent from the high-resolution NMR structures of Trx-$(SH)_2$ (Jeng *et al.*, 1994) and, indeed, would be difficult to form due to the van der Waals interaction between the two sulfur atoms of Cys 32 and Cys 35 in Trx-$(SH)_2$. The absence of the Cys 32 S−Cys 35 NH hydrogen bond is confirmed by the hydrogen-exchange behavior of *E. coli* thioredoxin (Jeng & Dyson, 1995), which shows over 5 orders of magnitude faster exchange rate of the Cys 35 amide proton in Trx-$(SH)_2$ compared to the rate seen for Trx-S_2.

The work described in this paper leads to the suggestion of a third possible mechanism for stabilization of the thiolate anion: sharing of the Cys 35 thiol proton between the two sulfur atoms, one of which is nominally protonated and the other deprotonated. Proton sharing between the two catalytic aspartic acid residues has been inferred in the active site of aspartic proteases (Pearl & Blundell, 1984; Davies, 1990), on the basis of a bell-shaped pH−rate profile, although more recent studies of the HIV protease−inhibitor complexes

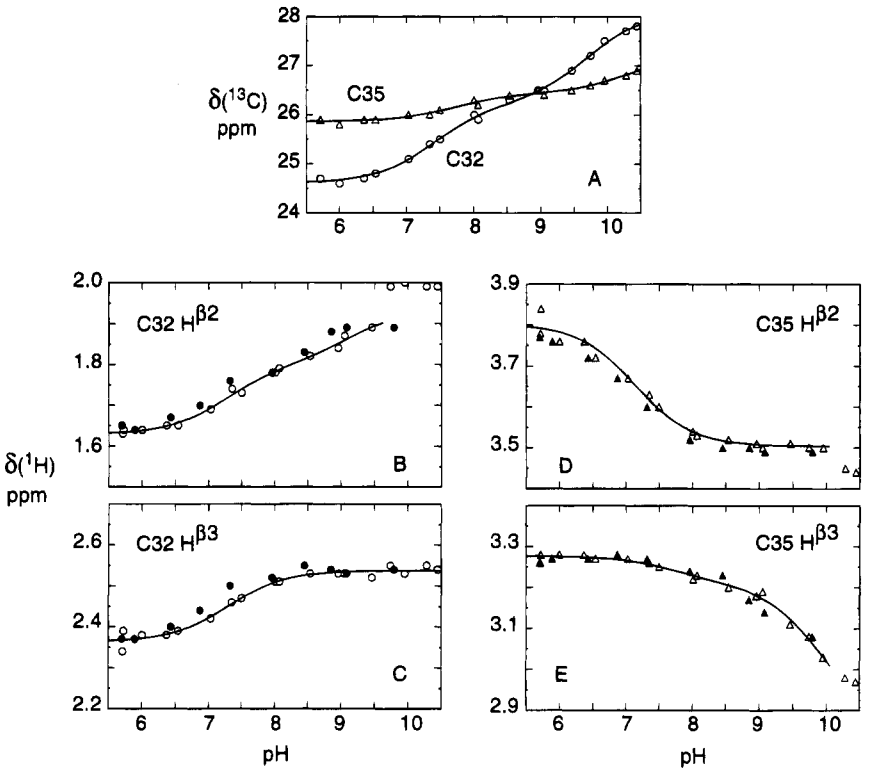

FIGURE 2: pH dependence of the chemical shifts of the C^{β} and H^{β} resonances obtained from HSQC spectra at 500 and 600 MHz, 298 K. Filled points represent data obtained previously at 308 K from ^1H measurements alone (Dyson *et al.*, 1991). Solid lines represent least-squares fits to the data using the program Templegraph. (A) Titration of $^{13}C^{\beta}$ resonances of Cys 32 and Cys 35, fitted using two pK_as. (B) Titration of Cys 32 $H^{\beta 2}$, fitted using two pK_as. The last four points are highly ambiguous due to resonance overlap and have been omitted from the fit. (C) Titration of Cys 32 $H^{\beta 3}$, fitted using one pK_a. (D) Titration of Cys 35 $H^{\beta 2}$, fitted using one pK_a. The last two points were omitted from the fit (see text). (E) Titration of Cys 35 $H^{\beta 2}$, fitted using two pK_as. The last two points were omitted from the fit (see text).

Table 1: pK_a Values Obtained from Curve Fitting of NMR Chemical Shifts as a Function of pH

	chemical modification[a]			NMR titrations			
				^1H[c] (308 K)		^{13}C (298 K)	
residue	pK_a	atom	δ (ppm)[b]	pK_a	$\Delta\delta$[d] (ppm)	pK_a	$\Delta\delta$ (ppm)
Cys 32	6.7	C^{β}	24.3			7.4 ± 0.1[e]	1.6
						9.7 ± 0.1	1.9
		$H^{\beta 2}$	1.67	6.8	0.13	7.5 ± 0.1	0.17
				8.4	0.15	9.1 ± 0.3	0.14
		$H^{\beta 3}$	2.37	6.8	0.18	7.3 ± 0.1	0.17
Cys 35	~9.0	C^{β}	25.9			7.7 ± 0.1	0.5
						(10.3 ± 0.3)[f]	(0.8)
		$H^{\beta 2}$	3.79	7.1	0.27	7.1 ± 0.1	0.30
		$H^{\beta 3}$	3.29			7.7 ± 0.3	0.07
				8.5	0.13	9.9 ± 0.3	0.35

[a] Data from Kallis and Holmgren (1980). [b] δ = chemical shift of ^{13}C or ^1H. [c] Data from Dyson *et al.* (1991). [d] $\Delta\delta$ = difference in chemical shift between the forms differing by a single proton that are linked by the given pK_a; data at pH 5.7 from Dyson *et al.* (1989) and Chandrasekhar *et al.* (1994). [e] Errors were estimated from the standard errors calculated as the sum of squares of residuals by the program Templegraph. [f] pK_a not well determined by data.

indicate that it may not necessarily be important in the catalytic mechanism, since the protonation state of the catalytic aspartic acid residues in these systems remains the same in the pH range 2−7 (Yamazaki *et al.*, 1994). A shared proton has also been postulated in myoglobin to explain histidine pK_as (Bashford *et al.*, 1993) and in bacteriorhodopsin for aspartate pK_as (Bashford & Gerwert, 1992; Sampogna & Honig, 1994). A recent NMR study of ribonuclease HI (Oda *et al.*, 1994) showed evidence from titration behavior of the active site aspartic acid residues that two of them shared a proton in the pH range where the enzyme is active. A common feature of these systems is the observation that the apparent pK_as of the titrating groups are often shifted, in many cases in opposite directions. For thioredoxin, the pK_as of the two thiols are demonstrably shifted in opposite directions, giving one apparent pK_a ~1 pH unit lower than normal, and one higher by the same amount. The high-resolution solution structures of *E. coli* Trx-$(SH)_2$ provide some corroboration for proton sharing between the two sulfur atoms. A "hydrogen-bonding" interaction where the Cys 35 thiol proton is positioned between the two sulfur atoms was observed in several of the solution structures of Trx-$(SH)_2$ (Jeng *et al.*, 1994) but could not be definitively distinguished from other alternatives involving apparent S−H···O=C hydrogen bonds, since no direct structural information is available for the thiol proton.

If the proton is indeed shared between the two sulfur atoms, giving a stabilized thiolate anion at neutral pH, an essential feature for the rapid initiation of the reduction reaction with protein substrates is now present. The mechanism shown in Figure 3 is essentially the same as that originally postulated by Kallis and Holmgren (1980), with the addition of the shared-proton concept. Following binding of the protein substrate P to the hydrophobic patch surrounding the active site in Trx-$(SH)_2$ (Eklund *et al.*, 1984), a noncovalent complex is formed, with the reacting groups in a highly hydrophobic environment between the two proteins. The initial step in the reaction of Trx-$(SH)_2$ with the disulfide of P is the nucleophilic attack of the thiolate anion of Cys 32 on the disulfide, with transient formation of a mixed disulfide between thioredoxin and P. Due to the proximity

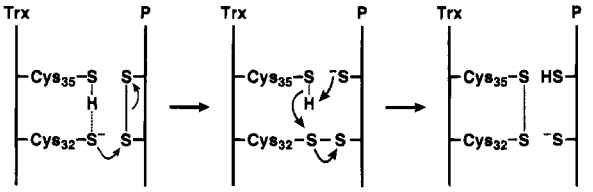

FIGURE 3: Schematic diagram of the mechanism of reduction of protein disulfides by Trx-$(SH)_2$, based on that suggested by Kallis and Holmgren (1980), incorporating the shared proton inferred from the present NMR study as a stabilizing interaction for the thiolate anion of Cys 32 prior to the initial nucleophilic attack on the disulfide of the substrate.

of the reacting cysteine sulfur atoms in this complex, it would be expected to be extremely short-lived, decomposing in the manner shown by a cyclic movement of electrons and the transfer of the shared proton to the newly formed P thiol. The completion of the reaction requires the dissociation of the final products, Trx-S_2 and reduced P. The residual negative charge on one of the P thiols, shown in the final step of the mechanism in Figure 3, should act to increase the dissociation rate, since its presence would be unfavorable in the hydrophobic environment of the complex. The advantage of the shared-proton concept in this scheme is that the negative charge on the reacting Cys 32 in the initial step, which would normally be a bar to formation of the Trx-$(SH)_2$–substrate complex, would be stabilized and delocalized by the interaction with the Cys 35 thiol.

This scheme does not take into account the probable roles of nearby buried charged residues in proton control before, during, and after both reduction and oxidation of thioredoxin. pH control is crucial for the oxidation of substrates by Trx-S_2, the reaction most similar to those of protein disulfide isomerases such as DsbA. The pK_a of the buried Asp 26 in Trx-$(SH)_2$ is 7.4 (Dyson *et al.*, 1991; M.-F. Jeng and H. J. Dyson, unpublished observations). This value is very close to that of the low-pK_a cysteine thiol, an indication that it may well be playing a role in the mechanism of the redox reactions of *E. coli* thioredoxin. These reactions are being explored through NMR studies of a series of mutants (M.-F. Jeng, A. Holmgren, and H. J. Dyson, manuscript in preparation), including those that mimic the active site of mammalian protein disulfide isomerase (Krause *et al.*, 1991). Subtle differences in the electrostatic interactions, related particularly to the proximity of charged groups to the active site, have been observed between thioredoxin and DsbA in recent calculations (Gane *et al.*, 1995) and used to explain differences in redox potential between the two systems. In particular, the histidine residue in DsbA that replaces Pro 34 of thioredoxin may disfavor the reduction reaction shown in Figure 3 by altering the charge balance at the active site. Indirect evidence for this hypothesis comes from the behavior of Trx-$(SH)_2$ with bovine pancreatic trypsin inhibitor (BPTI): little or no reaction occurs between these two proteins (A. Holmgren, unpublished observations), despite the presence of the exposed 14–38 disulfide, probably due to the close proximity of Lys 15, the functional lysine residue in BPTI. The net result of differences in redox potential between DsbA and thioredoxin is the preference of DsbA for oxidation of substrates to form disulfides compared to the equal facility of thioredoxin in oxidation or reduction reactions. For both proteins, reactivity is apparently largely governed by local electrostatic interactions, the key to which is proton control in the active site region. The indications reported here of proton sharing between the two cysteine residues in reduced thioredoxin may well prove to be an important component in the understanding of all of these systems.

ACKNOWLEDGMENT

We thank Monica Lindell for the preparation of the ^{13}C-labeled thioredoxin and P. E. Wright, D. Bashford, B. Honig, D. A. Case, and S. B. H. Kent for valuable discussions and critical comments on the manuscript and the ideas contained herein.

REFERENCES

Bashford, D., & Gerwert, K. (1992) *J. Mol. Biol. 224*, 473–486.
Bashford, D., Case, D. A., Dalvit, C., Tennant, L. L., & Wright, P. E. (1993) *Biochemistry 32*, 8045–8056.
Bax, A., Ikura, M., Kay, L. E., Torchia, D. A., & Tschudin, R. (1990) *J. Magn. Reson. 86*, 304–318.
Chandrasekhar, K., Krause, G., Holmgren, A., & Dyson, H. J. (1991) *FEBS Lett. 284*, 178–183.
Chandrasekhar, K., Campbell, A. P., Jeng, M.-F., Holmgren, A., & Dyson, H. J. (1994) *J. Biomol. NMR 4*, 411–432.
Davies, D. R. (1990) *Annu. Rev. Biophys. Biophys. Chem. 19*, 189–215.
Dyson, H. J., Holmgren, A., & Wright, P. E. (1989) *Biochemistry 28*, 7074–7087.
Dyson, H. J., Gippert, G. P., Case, D. A., Holmgren, A., & Wright, P. E. (1990) *Biochemistry 29*, 4129–4136.
Dyson, H. J., Tennant, L. L., & Holmgren, A. (1991) *Biochemistry 30*, 4262–4268.
Eklund, H., Cambillau, C., Sjöberg, B.-M., Holmgren, A., Jörnvall, H., Höög, J.-O., & Brändén, C.-I. (1984) *EMBO J. 3*, 1443–1449.
Forman-Kay, J. D., Clore, G. M., Wingfield, P. T., & Gronenborn, A. M. (1991) *Biochemistry 30*, 2685–2698.
Gane, P. J., Freedman, R. B., & Warwicker, J. (1995) *J. Mol. Biol.* (in press).
Holmgren, A. (1985) *Annu. Rev. Biochem. 54*, 237–271.
Holmgren, A., Söderberg, B.-O., Eklund, H., & Brändén, C.-I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2305–2309.
Jeng, M.-F., & Dyson, H. J. (1995) *Biochemistry 34*, 611–619.
Jeng, M.-F., Campbell, A. P., Begley, T., Holmgren, A., Case, D. A., Wright, P. E., & Dyson, H. J. (1994) *Structure 2*, 853–868.
Kallis, G. B., & Holmgren, A. (1980) *J. Biol. Chem. 255*, 10261–10265.
Katti, S. K., LeMaster, D. M., & Eklund, H. (1990) *J. Mol. Biol. 212*, 167–184.
Krause, G., Lundström, J., Lopez-Barea, J., Pueyo de la Cuesta, C., & Holmgren, A. (1991) *J. Biol. Chem. 266*, 9494–9500.
Langsetmo, K., Fuchs, J. A., & Woodward, C. (1991) *Biochemistry 30*, 7603–7609.
Li, H., Hanson, C., Fuchs, J. A., Woodward, C., & Thomas, G. J., Jr. (1993) *Biochemistry 32*, 5800–5808.
Lindley, H. (1960) *Biochem. J. 74*, 577–584.
Martin, J. L., Bardwell, J. C. A., & Kuriyan, J. (1993) *Nature 365*, 464–468.
Merutka, G., Dyson, H. J., & Wright, P. E. (1995) *J. Biomol. NMR 5*, 14–24.
Norwood, T. J., Boyd, J., Heritage, J. E., Soffe, N., & Campbell, I. D. (1990) *J. Magn. Reson. 87*, 488–501.
Oda, Y., Toshio, Y., Nagayama, K., Kanaya, S., Kuroda, Y., & Nakamura, H. (1994) *Biochemistry 33*, 5275–5284.
Pearl, L., & Blundell, T. (1984) *FEBS Lett. 174*, 96–101.
Sampogna, R. V., & Honig, B. (1994) *Biophys. J. 66*, 1341–1352.
Yamazaki, T., Nicholson, L. K., Torchia, D. A., Wingfield, P., Stahl, S. J., Kaufman, J. D., Eyermann, C. J., Hodge, C. N., Lam, P. Y. S., Ru, Y., Jadhav, P. K., Chang, C.-H., & Weber, P. C. (1994) *J. Am. Chem. Soc. 116*, 10791–10792.

BI951056M