Atassi, M. Z., & Singhal, R. P. (1970) *J. Biol. Chem. 245*, 5122–5128.
Björk, I., & Tanford, C. (1971) *Biochemistry 10*, 1271–1280.
Brown, N. C., Canellakis, Z. N., Lunding, B., Reichard, P., & Thelander, L. (1969) *Eur. J. Biochem. 9*, 561–573.
Chamberlin, M. (1974) *J. Virol. 14*, 509–516.
Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.
Holmgren, A. (1968) *Eur. J. Biochem. 6*, 475–484.
Holmgren, A. (1972a) *FEBS Lett. 24*, 351–354.
Holmgren, A. (1972b) *J. Biol. Chem. 247*, 1992–1998.
Holmgren, A. (1973) *J. Biol. Chem. 248*, 4106–4111.
Holmgren, A. (1980) in *Dehydrogenases Requiring Nicotinamide Coenzymes* (Jeffrey, J., Ed.) Birkhaüser Verlag, Basel (in press).
Holmgren, A., & Reichard, P. (1967) *Eur. J. Biochem. 2*, 187–196.
Holmgren, A., & Söderberg, B.-O. (1970) *J. Mol. Biol. 54*, 387–390.
Holmgren, A., & Sjöberg, B. M. (1972) *J. Biol. Chem. 247*, 4160–4164.
Holmgren, A., & Roberts, G. (1976) *FEBS Lett. 71*, 261–265.
Holmgren, A., & Slabý, I. (1979) *Biochemistry* (following paper in this issue).
Holmgren, A., Söderberg, B. O., Eklund, H., & Brändén, C. I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2305–2309.
Holmgren, A., Ohlsson, I., & Grankvist, M. L. (1978) *J. Biol. Chem. 253*, 430–436.
Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 317–330.
Mark, D., & Richardson, C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 780–784.
Mark, D. F., Chase, J. W., & Richardson, C. C. (1977) *Mol. Gen. Genet. 155*, 145–152.
Ornstein, L. (1964) *Ann. N.Y. Acad. Sci. 121*, 321–349.
Richards, F. M. (1958) *Proc. Natl. Acad. Sci. U.S.A. 44*, 162–167.
Slabý, I., & Holmgren, A. (1975) *J. Biol. Chem. 250*, 1340–1347.
Stryer, L., Holmgren, A., & Reichard, P. (1967) *Biochemistry 6*, 1016–1020.
Thelander, L. (1967) *J. Biol. Chem. 242*, 852–859.
Thelander, L. (1974) *J. Biol. Chem. 249*, 4858–4862.

Thioredoxin-C′: Mechanism of Noncovalent Complementation and Reactions of the Refolded Complex and the Active Site Containing Fragment with Thioredoxin Reductase†

Arne Holmgren* and Ivan Slabý‡

ABSTRACT: Thioredoxin-C′ is a refolded complex of the two inactive fragments thioredoxin-C-(1–37) and thioredoxin-C-(38–108) obtained by CNBr cleavage at the single Met-37 of thioredoxin from *Escherichia coli*. Thioredoxin-C′-S_2 is a weak complex that dissociates during gel chromatography, during gel electrophoresis, or in activity measurements as a substrate for NADPH and thioredoxin reductase. The association of the peptide fragments to thioredoxin-C′-S_2 and its dissociation were both rapid processes with half-times of 1–2 min at 10^{-6} M. Variation of the relative proportion of the fragments in the assay of thioredoxin-C′ with thioredoxin reductase gave an apparent K_D for thioredoxin-C′-S_2 of 2×10^{-6} M at 25 °C. Thioredoxin-C′-S_2 was a good substrate for NADPH and thioredoxin reductase and was calculated to have more than 50% relative activity when compared with thioredoxin-S_2. In contrast, thioredoxin-C′-$(SH)_2$ was inactive as a hydrogen donor for *E. coli* ribonucleotide reductase or as an insulin disulfide reductase, strongly suggesting that Met-37 is essential for the conformational change on oxidoreduction of thioredoxin. The active-site disulfide in thioredoxin-C-(1–37) was not reduced by NADPH and thioredoxin reductase; instead, the fragment was an inhibitor of thioredoxin reductase in the presence of NADPH and DTNB, which suggested nucleated folding of the fragment to generate a binding site for thioredoxin reductase. Structure–function relationships for chemically modified thioredoxin-C-(1–37) suggest essential functions for Met-37 and Lys-36 of thioredoxin in the interactions with thioredoxin reductase and ribonucleotide reductase. A model, based on the results with thioredoxin-C′, is presented to describe the three-dimensional complementarity of thioredoxin and thioredoxin reductase.

The three-dimensional structure of the oxidized form of *Escherichia coli* thioredoxin (thioredoxin-S_2)[1] has a high content of secondary structure that includes a central core of five strands of β-pleated sheet surrounded by four α helices (Figure 1) (Holmgren et al., 1975). The residues involved in the active center 14-membered disulfide bridge of the molecule form a protrusion between one of the helices and the middle strand of the pleated sheet. Thioredoxin has only one residue each of methionine (Met-37) and arginine (Arg-73) in its polypeptide chain of 108 amino acid residues. This has enabled

† From the Department of Chemistry, Karolinska Institutet, S-104 01 Stockholm 60, Sweden. *Received June 27, 1979; revised manuscript received September 26, 1979.* This investigation was supported by grants from the Swedish Medical Research Council Projects 13X-3529 and 13P-4292, Magnus Bergvalls Stiftelse, and by a short-term EMBO fellowship to I.S.

‡ Present address: Department of Medical Chemistry, Charles University, Karlovarská 48, Plzen, Czechoslovakia.

[1] Abbreviations used: thioredoxin-S_2 (T-S_2) and thioredoxin-$(SH)_2$ [T-$(SH)_2$], the oxidized and reduced forms of thioredoxin, respectively; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid). Peptide fragments have been designed by an adoption of the rules of the IUPAC–IUB Commission on Biochemical Nomenclature. Fragments obtained after CNBr cleavage are denoted by C and after selective cleavage with trypsin are denoted by T. The reconstituted noncovalent complexes are denoted thioredoxin-C′ and thioredoxin-T′ (Slabý & Holmgren, 1975).

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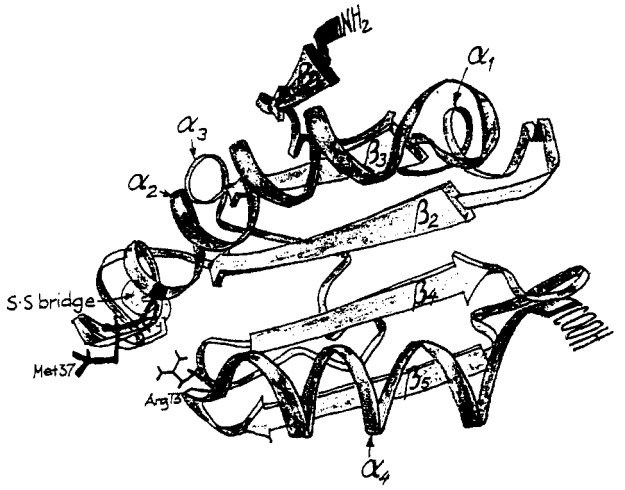

FIGURE 1: Schematic drawing of the three-dimensional structure of *E. coli* thioredoxin-S_2 (Holmgren et al., 1975). The positions of Met-37 and Arg-73 are indicated in the structure. The positions of the S–S bridge (Cys-32 to Cys-35) and the five strands of β-pleated sheet (β_1–β_5) and the four α helices (α_1–α_4) are also shown.

the selective cleavage of the protein either at the methionine residue with cyanogen bromide or at the arginine residue with trypsin after reversible blocking of all lysine residues with citraconic anhydride (Holmgren & Reichard, 1967; Slabý & Holmgren, 1975). In each case the two corresponding separated peptide fragments when mixed are capable of refolding by complementation to give thioredoxin-like structures (Holmgren, 1972; Slabý & Holmgren, 1975). Thioredoxin-T′ consists of thioredoxin-T-(1–73) and thioredoxin-T-(74–108) in a 1:1 complex. Thioredoxin-C′ consists of thioredoxin-C-(1–37), which has a COOH-terminal homoserine residue in place of Met-37, in complex with thioredoxin-C-(38–108).

Thioredoxin-T′ is a stable complex with an estimated K_D lower than 10^{-8} M (Slabý & Holmgren, 1979). Furthermore, it has physicochemical properties similar to thioredoxin in its oxidized and reduced form (Slabý & Holmgren, 1979). However, thioredoxin-T′-S_2 exhibited only low activity as a substrate for thioredoxin reductase when compared with native thioredoxin-S_2. This low relative activity of thioredoxin-T′ was the result of an around 100-fold increase in the apparent K_m value of the molecule in the reaction with thioredoxin reductase. The reduced form of thioredoxin-T′ exhibited a low but significant activity as a substrate for ribonucleotide reductase. The discontinuity of the peptide chain in thioredoxin, at Arg-73, thus permitted the main folding interactions of the native molecule, but it has a dramatic effect on the catalytic functions of the active center.

In this study the structure and catalytic activity of thioredoxin-C′ have been examined. The thioredoxin-C′-S_2 complex has high activity as substrate for thioredoxin reductase but is inactive with ribonucleotide reductase. The mechanism of complex formation has been analyzed, and thioredoxin-C′ is shown to be a weak complex. The NH_2-terminal fragment, thioredoxin-C-(1–37), which contains the active-site disulfide, slowly inactivates thioredoxin reductase in the presence of NADPH and DTNB. This suggests the presence of a binding site for thioredoxin reductase in the 1–37 region of thioredoxin-S_2. A model for the binding of thioredoxin-S_2 to thioredoxin reductase based on the results with the two complementing systems is presented.

Experimental Procedure

If not otherwise indicated, materials and methods were as specified in the preceding paper (Slabý & Holmgren, 1979) or as described previously (Slabý & Holmgren, 1975).

Preparation of Thioredoxin-C-(1–37) and Thioredoxin-C-(38–108). Thioredoxin was cleaved with cyanogen bromide in 70% formic acid for 24 h at 25 °C (Holmgren & Reichard, 1967). Separation of the resulting peptide fragments from each other and uncleaved thioredoxin was performed on a column of Sephadex G-50 equilibrated with 50% acetic acid at 25 °C. After evaporation of the acetic acid by repeated lyophilizations, the two fragments thioredoxin-C-(1–37) and thioredoxin-C-(38–108) were dissolved in 0.06 M NH_4HCO_3, pH 8.0. They were then rechromatographed on columns of Sephadex G-50 (0.9 × 130 cm) in 0.06 M NH_4HCO_3 to give single symmetrical peaks. Each rechromatographed fragment was shown to be pure by amino acid analysis (Holmgren & Reichard, 1967). After lyophilization, each fragment was stored as a 0.5–1 mM solution in 0.06 M NH_4HCO_3, pH 8.0, at −20 °C.

Modification of Amino Groups with Citraconic Anhydride. Thioredoxin-C-(1–37) and the thioredoxin-C-(38–108) were passed through columns of Sephadex G-25 equilibrated with 0.50 M potassium phosphate, pH 8.5. The modification of amino groups was done with citraconic anhydride as described (Slabý & Holmgren, 1975). The citraconylated peptides were isolated after Sephadex G-25 chromatography in 0.5% NH_4HCO_3, pH 7.9, and were stored frozen at −20 °C.

Amino Acid Analysis. Peptides were hydrolyzed with 6 M HCl for 24 h at 110 °C in vacuo. The amino acid composition of the hydrolysates was determined by using a Beckman 120B amino acid analyzer. Homoserine and homoserine lactone were analyzed as described by Ambler (1965).

Thioredoxin Assays. The experimental details for the three methods used to measure the catalytic activity of thioredoxin are given in the preceding paper (Slabý & Holmgren, 1979). *Method 1. DTNB Assay.* This measures the activity of thioredoxin-S_2 as a substrate for thioredoxin reductase. *Method 2. Insulin Assay.* This measures a protein disulfide reductase activity of thioredoxin and the activity of thioredoxin-S_2 as a substrate for thioredoxin reductase. *Method 3. Ribonucleotide Reductase Assay.* This shows the function of thioredoxin-$(SH)_2$ as a hydrogen donor for *E. coli* ribonucleotide reductase. The reaction involves the reduction of a catalytically active disulfide in the enzyme.

Protein Determination. The concentration of peptides was determined by reading the absorbance at 280 and 310 nm in a Zeiss PMQ 3 spectrophotometer. The molar absorptivity for native thioredoxin and thioredoxin-C′ was 13 700 M^{-1} cm^{-1}; it was 10 000 M^{-1} cm^{-1} for thioredoxin-C-(1–37) and 3700 M^{-1} cm^{-1} for thioredoxin-C-(38–108) (Holmgren & Reichard, 1967).

Gel Electrophoresis. Polyacrylamide disc electrophoresis was performed with a Shandon SAE 2734 apparatus using the discontinuous buffer system at pH 8.9 as described by Ornstein (1964) and Davis (1964). The gels were stained with 0.25% Coomassie brilliant blue in 12% trichloroacetic acid.

Quantitative Immunoprecipitation Reactions. Rabbit antithioredoxin γ-globulin (50 μL) was incubated with increasing amounts of antigen (Holmgren & Sjöberg, 1972).

Carboxypeptidase Digestion of Thioredoxin-C-(1–37). Thioredoxin-C-(1–37), 400 μL of 5 × 10^{-4} M in 0.06 M NH_4HCO_3, pH 7.8, was incubated for 18 h at 37 °C with 20 μg of carboxypeptidase A (CoADFP, Worthington Biochemical Corp.). Amino acid analysis showed the release of only homoserine in 90% yield and no lysine. Peptide-C-(1–36) was isolated free from carboxypeptidase by chromatography on Sephadex G-50 (1.5 × 25 cm) in 0.06 M NH_4HCO_3. The

Table I: K_{AV} Values for the Peptide Fragments and Thioredoxin-C′ Determined by Gel Chromatography[a]

sample	K_{AV}
thioredoxin-C-(1–37)	0.50[b]
thioredoxin-C-(38–108)	0.20
thioredoxin-C′	0.26
thioredoxin-S_2	0.34

[a] A column of Sephadex G-50 (0.9 × 140 cm) equilibrated with 0.06 M NH_4HCO_3, pH 8.0, at 25 °C was used. K_{AV} values were calculated from the equation (Laurent & Killander, 1964) $K_{AV} = (V_e - V_0)/(V_T - V_0)$, where V_e is elution volume of protein, V_T is the total volume of the column, and V_0 is one-third of V_T. [b] This peptide may be retarded on Sephadex due to its content of two tryptophan residues. The K_{AV} value may thus be overestimated.

Table II: Amino Acid Composition of Thioredoxin-C′

amino acid	residues/mol	
	thioredoxin-C′	thioredoxin[a]
lysine	10.90	10
histidine	1.16	1
arginine	1.00	1
aspartic acid	14.80	15
threonine	5.47	6
serine	2.68	3
glutamic acid	7.87	8
proline	5.26	5
glycine	9.29	9
alanine	11.66	12
valine	4.76	5
homoserine[b]	0.90	1
isoleucine	8.03	9
leucine	12.54	13
tyrosine	1.87	2
phenylalanine	3.55	4
half-cystine	1.85	2
tryptophan[c]	2.0	2

[a] From the amino acid sequence (Holmgren, 1968). [b] Calculated as the sum of homoserine and homoserine lactone. [c] Estimated spectrophotometrically from the molar extinction coefficient at 280 nm.

amino acid composition showed the expected composition, with the presence of 2.8 residues of lysine and no homoserine. After lyophilization, thioredoxin-C-(1–36) was dissolved in 0.06 M NH_4HCO_3 at 2.8 × 10^{-4} M and stored frozen at −20 °C.

Results

Properties of Thioredoxin-C′. The two pure peptide fragments, thioredoxin-C-(1–37) and thioredoxin-C-(38–108), were chromatographed separately on a calibrated column of Sephadex G-50. They showed single, sharp, symmetrical peaks with K_{AV} values indicating random-coil structures when compared with the K_{AV} value of native thioredoxin on the same column (Table I). Thioredoxin-C′, formed by incubation of equimolar amounts of thioredoxin-C-(1–37) and thioredoxin-C-(38–108), showed three main peaks of protein (I–III), when chromatographed on Sephadex G-50. The middle peak (II) was identified as thioredoxin-C′ by its amino acid composition (Table II) that was the sum of a 1:1 complex of the two fragments. Furthermore, this peak had the same immunoprecipitation activity as native thioredoxin (Holmgren, 1972). The two other peaks (I and III) consisted of uncomplexed thioredoxin-C-(38–108) and thioredoxin-C-(1–37). This was confirmed by their ultraviolet spectra, their nonidentity with native thioredoxin in immuno double diffusion, and their amino acid compositions. The low yield of the complex suggested that either only a fraction of the peptides could generate thioredoxin-C′ or thioredoxin-C′ was dissociated into peptide fragments during the chromatography. The last interpretation, consistent with a weak complex, was confirmed by rechromatography of the thioredoxin-C′ (peak II). This resulted in generation of free peptides and a pattern similar to that seen in the first chromatography. The K_{AV} value for thioredoxin-C′ was significantly lower than that of thioredoxin-S_2, consistent with a larger Stokes radius of the complex.

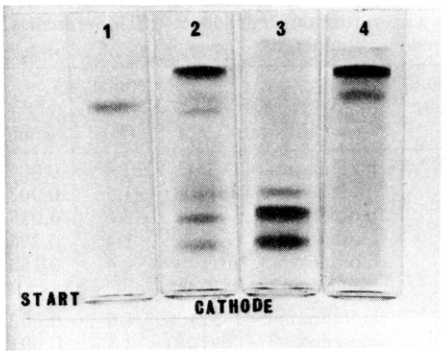

FIGURE 2: Polyacrylamide gel electrophoresis of thioredoxin-C′. 5 μg of thioredoxin-S_2 (1) and 10 μg each of thioredoxin-C′ (2), thioredoxin-C-(38–108) (3), and thioredoxin-C-(1–37) (4) were run in native gels at pH 8.9. For experimental details, see Slabý & Holmgren (1979).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis at pH 8.9 in the absence of denaturants showed that thioredoxin-C′ was almost totally dissociated into thioredoxin-C-(1–37) and thioredoxin-C-(38–108) during the electrophoresis (Figure 2). The same pattern was observed by electrophoresis at pH 6.0, where the complex also was largely dissociated. Thioredoxin-C-(1–37) gave a single band on electrophoresis in a position corresponding to its acidic character. In contrast, thioredoxin-C-(38–108) was separated into two main bands and a faint third band. These molecular forms represented differences in charges since they could also be prepared by chromatography on a column of DEAE-cellulose equilibrated with 0.01 M Tris-HCl, pH 8.74, eluted with a linear gradient of Tris-HCl from 0.01 to 0.20 M Tris-HCl. Both forms of thioredoxin-C-(38–108) were equally active in generating enzymatically active thioredoxin-C′. The result is consistent with partial deamidation of Asn-83 during the acid chromatographic preparation of thioredoxin-C-(38–108) (Holmgren, 1968). The possibility that the presence of two bands in the thioredoxin-C-(38–108) preparation was the result of partial dimerization was ruled out by the identical electrophoresis pattern in gels containing 7 M urea. These results also showed that the peptide fragments behaved as random coils in solution.

Formation of a Covalent Complex in Thioredoxin-C′. The peptide backbone may be resynthesized in a cyanogen bromide cleaved protein through direct coupling of homoserine lactone and the amino group (Dyckes et al., 1973). The results of the gel electrophoresis (Figure 2) of thioredoxin-C′ showed no evidence for any covalent complex. Incubation of thioredoxin-C′ at pH 8.0 for 24 h, followed by gel electrophoresis, did not reveal any appreciable amount of covalent complex. During cyanogen bromide cleavage of thioredoxin, some covalent complex is formed which contains homoserine and no methionine (Holmgren & Reichard, 1967).

Enzymatic Activity of Thioredoxin-C′-S_2 with Thioredoxin Reductase. Thioredoxin reductase from *E. coli* uses NADPH to reduce the disulfide of thioredoxin-S_2 from *E. coli* (reaction 1). The reduction of DTNB by thioredoxin-$(SH)_2$ is the basis

Table III: Concentration Dependence of Thioredoxin-C′ Activity with Thioredoxin Reductase

thioredoxin reductase (M)	substrate	concn × 10^6 (M)	act.[a] (ΔA_{412} × min^{-1}) (%)
4×10^{-8}	thioredoxin-C-(1-37)	2.0	−0.001
	thioredoxin-C-(38-108)	1.5	0.001
	thioredoxin-C′ [b]	2.0	0.057 (13.5)
	thioredoxin-S_2	1.4	0.295 (100)
	thioredoxin-S_2 after acid treatment[c]	1.4	0.155 (53)
2×10^{-8}	thioredoxin-C-(1-37)	4.0	−0.003
	thioredoxin-C-(38-108)	3.8	−0.001
	thioredoxin-C′ [b]	4.0	0.054 (20.0)
	thioredoxin-S_2	2.8	0.190 (100)

[a] The activity was determined with the DTNB assay at 25 °C. The enzymatic activity was calculated from the change during the first and second minute. For details, see Experimental Procedure. [b] Thioredoxin-C′ was prepared by mixing thioredoxin-C-(1-37) and thioredoxin-C-(38-108), both at final concentrations of 1×10^{-4} M in 0.15 M NaCl-0.01 M potassium phosphate, pH 7.0, at 0 °C. The mixtures were incubated for 10 min before assay. [c] Control of thioredoxin-S_2 that had been incubated in 70% formic acid without cyanogen bromide and chromatographed on Sephadex G-50 in 50% acetic acid under the conditions used to prepare the peptide fragments.

for a rapid thioredoxin-S_2 assay with an excess of thioredoxin reductase (reaction 2):

$$\text{thioredoxin-}S_2 + \text{NADPH} + H^+ \xrightarrow{\text{thioredoxin reductase}} \text{thioredoxin-}(SH)_2 + \text{NADP}^+ \quad (1)$$

$$\text{thioredoxin-}(SH)_2 + \text{R-S-S-R} \xrightarrow{\text{spontaneous}} \text{thioredoxin-}S_2 + 2\text{R-SH} \quad (2)$$

where R is 2-nitrobenzoic acid. Only 1 mol of DTNB is consumed in the reaction with the two vicinal SH groups forming 2 mol of 5-thio-2-nitrobenzoic acid and regenerating the disulfide in thioredoxin.

Both peptide fragments were completely inactive in the DTNB assay (Table III). Thioredoxin-C′, formed by a short preincubation, showed from 10 to 20% of the activity of thioredoxin-S_2 at (2–4) × 10^{-6} M concentration (Table III). Several experiments showed that the relative activity of thioredoxin-C′ was increasing with concentration, consistent with a dissociating system of peptide fragments. Since a control of acetic acid treated thioredoxin-S_2 showed about 50% reduction in enzymatic activity (Table III), the activity of thioredoxin-C′-S_2 at 4×10^{-6} M is about 40% of that of native thioredoxin.

The association of the peptide fragments by preincubation to form thioredoxin-C′ was time dependent and also changed with ionic strength and temperature (data not shown). The inclusion of 8 M urea during preincubation at 1×10^{-4} M blocked the reaction. It was also possible to study the generation of enzyme activity in the DTNB assay mixture. As shown in Figures 3 and 4, the presence of one peptide fragment, at 5×10^{-6} M, and thioredoxin reductase gave no activity. The addition of a small volume of the complementing peptide fragment to give the same concentration gave a rapid increase in enzyme activity. The half-time for generation of activity was about 90 s, at 5×10^{-6} M, and the final level of activity was about 5% of that of native thioredoxin. As shown in Figure 3, a higher peptide concentration gave a faster regeneration of activity. Furthermore, the addition of one further aliquot of the complementing peptide rapidly established a new and higher rate. These observations are consistent with the

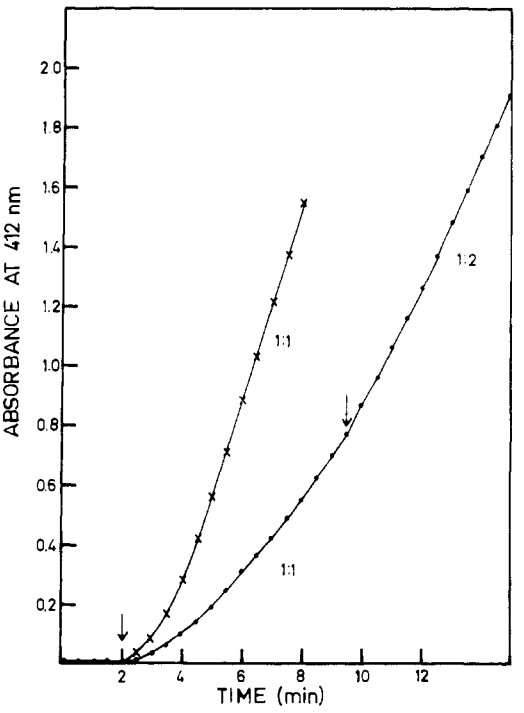

FIGURE 3: Enzymatic activity of thioredoxin-C-(38–108), 5.1×10^{-6} (●) and 8.3×10^{-6} M (×). One equivalent of thioredoxin-C-(1–37) was added at each arrow. The DTNB assay was used.

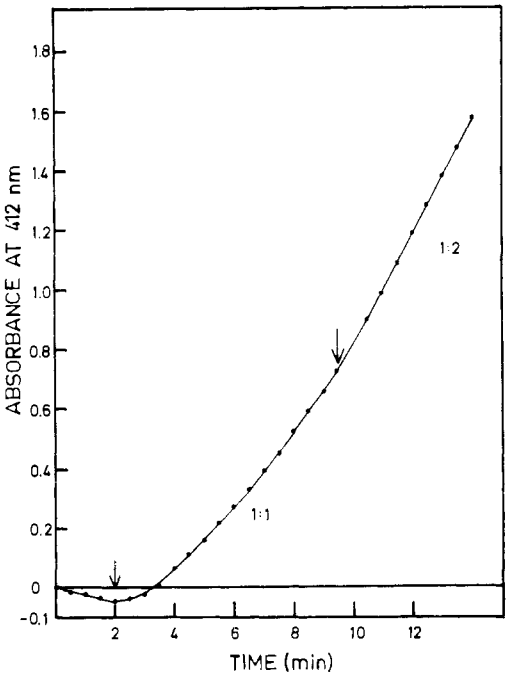

FIGURE 4: Enzymatic activity of thioredoxin-C-(1–37), 5.1×10^{-6} M. One equivalent of thioredoxin-C-(38–108) was added at each arrow. The DTNB assay was used.

reversible formation of active thioredoxin-C′-S_2 according to reaction 3:

$$\text{thioredoxin-C-(1-37)-}S_2 + \text{thioredoxin-C-(38-108)} \rightleftharpoons \text{thioredoxin-C′-}S_2 \quad (3)$$

The mechanism shown in reaction 3 was supported by a series of experiments indicating dissociation of thioredoxin-C′. Thioredoxin-C′ was formed by incubation at 3×10^{-4} M and subsequently diluted into DTNB assay cuvettes to give final concentrations of (1–10) × 10^{-6} M. This resulted in a decrease of activity (30–40% of that of native, initially) before a con-

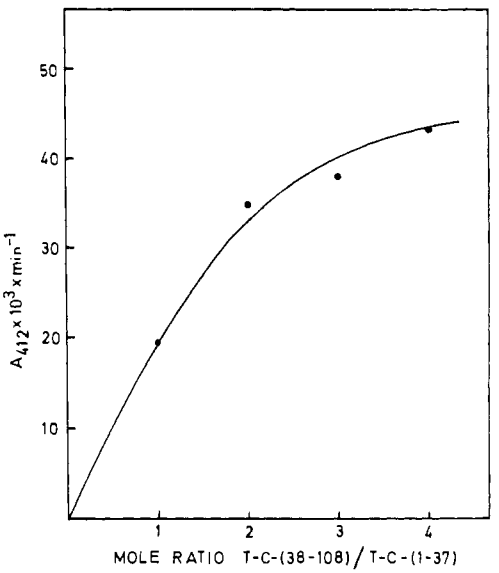

FIGURE 5: Rate of DTNB reduction as a function of the molar ratio of thioredoxin-C-(38–108) to thioredoxin-C-(1–37). The concentration of thioredoxin-C-(1–37) was 1.6×10^{-6} M.

stant rate was obtained. The half-time for the decrease was around 2 min at 5×10^{-6} M.

The activity measurements allowed the equilibrium constant, K, for reaction 3 to be estimated by the use of an equation derived for a complementing system in staphylococcal nuclease by Light et al. (1974):

$$K = \frac{a}{(1-a)(R-a)} \frac{1}{C_0} \quad (4)$$

where R is the molar ratio of thioredoxin-C-(38–108) to thioredoxin-C-(1–37), C_0 is the initial concentration of thioredoxin-C-(1–37), and a is the fraction of thioredoxin-C′ produced. When C_0 was kept constant at 1.6×10^{-6} or 3.2×10^{-6} M and the value of R was varied at 25 °C (see Figure 5), the value of K was 5×10^{5} M^{-1}, equivalent to a dissociation constant of 2×10^{-6} M. The activity of thioredoxin-C′ for $a = 1.00$ was estimated experimentally to be close to 50% of the activity for native thioredoxin. Furthermore, the covalent complex of thioredoxin containing homoserine in place of methionine isolated after CNBr treatment of the molecule (Holmgren & Reichard, 1967) gave 52% of the activity of native thioredoxin.

Catalytic Activity of Thioredoxin-C′-(SH)$_2$. When thioredoxin-C′-S_2 was mixed with NADPH and thioredoxin reductase, oxidation of 1 molar equiv of NADPH occurred. The formation of 2 mol of SH groups per mol of thioredoxin-C′ was determined with DTNB in 6 M guanidine hydrochloride. However, thioredoxin-C′-$(SH)_2$ had essentially no activity as a hydrogen donor for ribonucleotide reductase. It was also inactive as a disulfide reductase in the reduction of insulin disulfides (Table IV). This finding suggests that the structure of thioredoxin-C′-$(SH)_2$ does not permit the catalytic functions or that the complex falls apart due to weaker binding interactions as the result of a conformational change on reduction. However, the addition of an excess of one peptide fragment did not give any activity, making this explanation unlikely.

Inactivation of Thioredoxin Reductase by Thioredoxin-C′. The enzymatic rate in a DTNB assay of thioredoxin-C′ leveled off with time and was close to zero after 30 min. This could be due to inactivation of thioredoxin-C′ or of thioredoxin reductase. Addition of thioredoxin reductase restored the activity whereas addition of thioredoxin did not. This demonstrated that incubation of thioredoxin reductase with thioredoxin-C′ inactivated the enzyme. Further analyses of the possible inhibitory effect of the two peptide fragments on the DTNB assay with varying thioredoxin concentrations clearly showed that the inhibitory effect was due to peptide-C-(1–37) (Table V). In fact, the incubation of thioredoxin-C-(1–37) with NADPH, DTNB, and thioredoxin reductase resulted in a time- and concentration-independent inhibition of thioredoxin reductase (Table V). Addition of excess thioredoxin-C-(38–108) to fully inactivated enzyme was without effect.

Table IV: Catalytic Activity of Thioredoxin-C′-$(SH)_2$

addition	catalytic act. insulin reduction[a] (μM S-S min^{-1})	catalytic act. CDP reduction[b] [nmol/(dCDP 10 min)]
thioredoxin-C′, 1.7×10^{-5} M, pH 7.0	0.2 (0.1%)	
thioredoxin, 5.4×10^{-7} M, pH 7.0	18.6	
thioredoxin-C′, 1.7×10^{-5} M, plus thioredoxin, 5.4×10^{-7} M, pH 7.0	17.8	
thioredoxin-C′, 1.5×10^{-6} M		<0.1
thioredoxin-C′, 1.5×10^{-5} M		1.3 (0.5%)
thioredoxin-S_2, 4×10^{-7} M		7.1

[a] The assay was performed with 1.2×10^{-4} M insulin using 4×10^{-4} M NADPH and 2×10^{-7} M *E. coli* thioredoxin reductase at pH 7.0 in 0.1 M potassium phosphate–1 mM EDTA. The oxidation of NADPH was followed spectrophotometrically. The same result was obtained at pH 8.0 in 0.1 M Tris-HCl–1 mM EDTA. [b] Assay with 15 μg of *E. coli* ribonucleotide reductase, 5×10^{-4} M NADPH, and 5×10^{-7} M thioredoxin reductase. For details, see Slaby & Holmgren (1979).

Table V: Effect of the Peptide Fragments on Thioredoxin Reductase Activity in DTNB Reduction

preincubation[a]	act. $\Delta A_{412} \times min^{-1}$	act. %
control, 0.06 M NH_4HCO_3, 6 min	0.050	100
T-C-(38–108), 24×10^{-6} M, 6 min	0.052	104
T-C-(1–37), 28×10^{-6} M, 6 min	0.017	34
T-C-(1–37), 28×10^{-6} M, 3 min	0.035	50
T-C-(1–37), 100×10^{-6} M, 5 min	0.002	4

[a] The mixture contained in 500 μL the following: 0.10 M Tris-HCl–1 mM EDTA, pH 8.0, 0.4 mM NADPH, 0.4 mM DTNB, and 0.1 mg/mL bovine serum albumin at 25 °C. Thioredoxin reductase, 3 μL of 0.28 mg/mL (final concentration 2.5×10^{-8} M), was added to all cuvettes. The peptide fragments (5–30 μL) or 0.06 M NH_4HCO_3 was added to the cuvettes which were preincubated for the times shown. The enzymatic reaction was then started by addition of 10 μL of thioredoxin-S_2 (1.5×10^{-6} M final concentration) to all cuvettes, and the reaction was followed at 412 nm against a blank.

Inhibition of Thioredoxin Reductase by Thioredoxin-C-(1–37). In a series of experiments the effects of thioredoxin-C-(1–37) as an inhibitor of thioredoxin reductase in the DTNB assay were studied by using 1.0×10^{-5} M thioredoxin-C-(1–37), $(0.2–1) \times 10^{-5}$ M thioredoxin, and 6×10^{-9} M thioredoxin reductase. When thioredoxin and thioredoxin-C-(1–37) were added simultaneously no clear inhibition was observed. This shows that thioredoxin-C-(1–37) must be a weak competitive inhibitor and that thioredoxin reductase is protected by thioredoxin. However, preincubation of the enzyme with thioredoxin-C-(1–37), as shown in Table V, had a clear effect on the V_{max} of the enzyme, indicating some irreversible inactivation mechanism. It was treated with EDTA without effect to rule out contamination of thioredoxin-C-(1–37) with heavy metals. Addition of the reduced form of thioredoxin-C-(1–37) obtained by chemical reduction

Table VI: Effect of DTNB and NADPH on Inactivation of Thioredoxin Reductase by Thioredoxin-C-(1-37)

preincubation[a]	time (min)	act. (% of control)
T-C-(1-37), 7.5×10^{-5} M	20	9
T-C-(1-37), 7.5×10^{-5} M, minus NADPH	25	110
T-C-(1-37), 1.3×10^{-5} M, minus DTNB	20	120
T-C-(1-37), reduced form, 1.3×10^{-5} M	20	130
T-C-(1-37), reduced form, 1.3×10^{-5} M, plus DTNB and NADPH	20	34

[a] The conditions were the same as those described in Table V except that NADPH or DTNB was excluded from the preincubation mixture where indicated and added together with thioredoxin-S_2 at the start of the assay.

Table VII: Effects of Removal of Homoserine-37 in Thioredoxin-C-(1-37) on Thioredoxin-C'-S_2 Activity with Thioredoxin Reductase

substrate	act.[a] ($\Delta A_{412} \times \text{min}^{-1}$)
T-C-(1-36) plus T-C-(38-108), 3.7×10^{-6} M	−0.001
T-C-(1-37) plus T-C-(38-108), 3.7×10^{-6} M	0.016
thioredoxin-S_2, 3×10^{-7} M	0.048

[a] The DTNB assay was used.

to the DTNB assay mixture was shown to result in very rapid complete reoxidation of the two vicinal SH groups to a disulfide and unchanged inhibition. This reoxidation pattern of reduced thioredoxin-C-(1-37) was the same in 6 M guanidine hydrochloride. As shown in Table VI, the inhibitory effect was dependent on NADPH and DTNB and the oxidized form of thioredoxin-C-(1-37). These results strongly suggest that thioredoxin-C-(1-37) binds as a substrate analogue to the two-electron reduced form of thioredoxin reductase (Holmgren, 1980) and, together with DTNB, inactivates the enzyme.

Effects of Removal of Homoserine in Thioredoxin-C-(1-37). The COOH-terminal sequence of thioredoxin-C-(1-37) is Lys-Hse, where Hse is homoserine. Peptide-C-(1-36) was prepared by carboxypeptidase A digestion, which quantitatively removed the homoserine residue. The peptide was purified by Sephadex G-50 chromatography and tested for complementation activity by the immunoprecipitation assay using Ouchterlony immunodiffusion and enzymatic activity by the DTNB assay. The result demonstrated unchanged complementation activity in the immunoprecipitation assay (Slabý & Holmgren, 1975) whereas all activity in the DTNB assay for thioredoxin-C' was lost (Table VII).

Refolding of T-C' from Citraconylated Peptide Fragments. The amino groups of thioredoxin-C-(1-37) and thioredoxin-C-(38-108) were blocked by citraconylation as described for thioredoxin-S_2 (Slabý & Holmgren, 1975, 1979). Thioredoxin-C' was prepared by preincubation at 1×10^{-4} M from the various combinations of native and citraconylated peptide fragments. The formation of a complex was assayed by quantitative immunoprecipitation and by determination of enzymatic activity in the DTNB assay. The mixture of citraconylated thioredoxin-C-(1-37) and native thioredoxin-C-(38-108) gave the antigenic activity of thioredoxin-C' (Figure 6). This suggests that folding into thioredoxin-C' was possible. However, the enzymatic activity in the DTNB assay from citraconylated thioredoxin-C-(1-37) and native thioredoxin-C-(38-108) was zero ($\Delta A_{412} \times \text{min}^{-1}$, 0.008 vs. 0.156 for unmodified thioredoxin-C', both at 5×10^{-6} M). This shows that amino groups of thioredoxin-C-(1-37) play a major role in the catalytic interactions of thioredoxin-C' with thioredoxin reductase and DTNB.

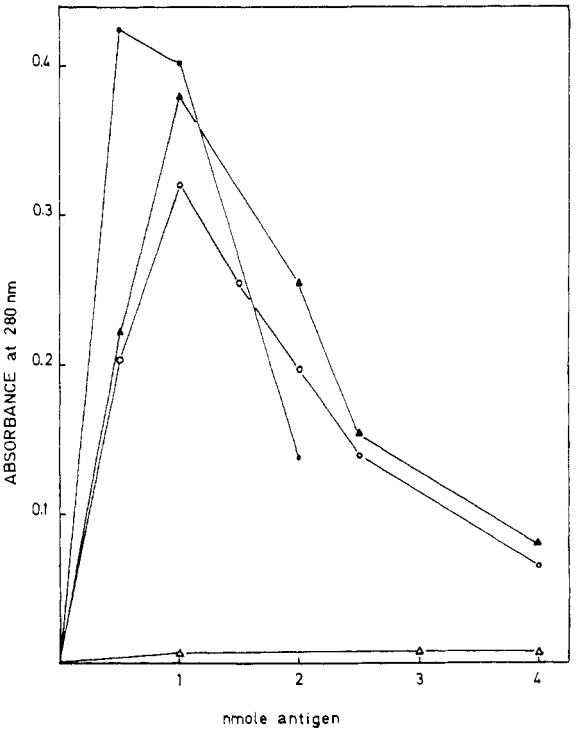

FIGURE 6: Quantitative immunoprecipitation reaction of thioredoxin (●), thioredoxin-C' (▲), a 1:1 mixture of thioredoxin-C-(38-108) and citraconylated thioredoxin-C-(1-37) (○), and a 1:1 mixture of citraconylated thioredoxin-C-(38-108) and thioredoxin-C-(1-37) (△). No precipitation occurred with both fragments citraconylated.

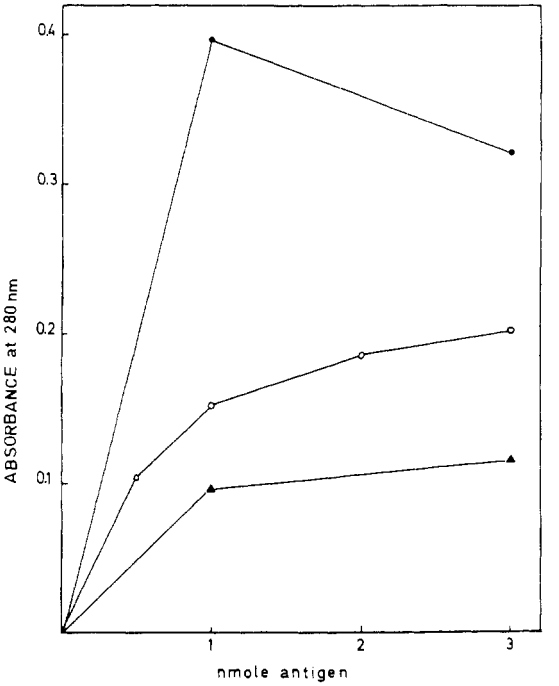

FIGURE 7: Quantitative immunoprecipitation reaction of thioredoxin-T' (●), thioredoxin-T-(1-73) (▲), and a 1:1 mixture of thioredoxin-T-(1-73) and citraconylated thioredoxin-T-(74-108) (○).

In contrast, citraconylated thioredoxin-C-(38-108) did not yield any immunoprecipitation or catalytic activity together with thioredoxin-C-(1-37) (Figure 6). The amino groups responsible for the lack of immunogenic activity may well be in the 74-108 part of thioredoxin-C-(38-108) since citraconylated thioredoxin-T-(74-108) did not restore immunoprecipitation with thioredoxin-T-(1-73) (Figure 7).

Table VIII: Comparison of Properties of the Thioredoxin-C′ and the Thioredoxin-T′ Complementing Systems[a]

parameter	T-T′	T-C′	T-T-(1-73)	T-T-(74-108)	T-C-(1-37)	T-C-(38-108)
complex stability	high, $K_D < 10^{-8}$	low, $K_D = 2 \times 10^{-6}$				
overall rate of complex formation	slow	fast				
crystallized as thioredoxin-S_2	yes	no				
rel act. with thioredoxin reductase	1-2% K_m increase	50%	none	none	inhibitory in DTNB assay	none
rel act. with ribonucleotide reductase	3-5%	inactive	none	none	none	none
antibody precipitation	full[b]	full[b]	yes, some[c]	none	none	yes, some[c]
inhibn of precipitation of thioredoxin-antithioredoxin			strong	strong	none	very strong

[a] Data on thioredoxin-T′ were from Slaby & Holmgren (1979). [b] Complete antigen with antithioredoxin. It has a zone of inhibition at antigen excess (Slaby & Holmgren, 1975). [c] Behaves as an incomplete antigen with no zone of inhibition (Slaby & Holmgren, 1975).

Radioimmunoassay of Thioredoxin-C′. The results of experiments with the radioimmunoassay for thioredoxin-S_2 (Slabý & Holmgren, 1979) applied to the peptide fragments and thioredoxin-C′ are shown in Figure 8. The peptide fragments give some competition at high concentration. The competition curve for thioredoxin-C′ indicates that there are some conformational differences between thioredoxin-C′ and thioredoxin-S_2.

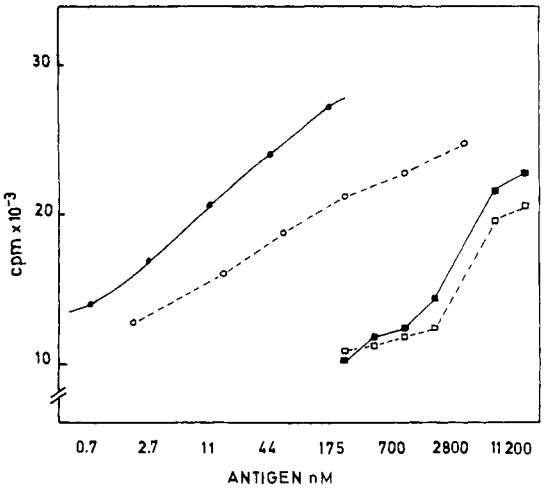

FIGURE 8: Double-antibody radioimmunoassay of native thioredoxin (●), thioredoxin-C′ (○), thioredoxin-C-(38–108) (■), and thioredoxin-C-(1–37) (□). The free [^{125}I]thioredoxin in 100 μL of the supernatant was plotted against the concentration of thioredoxin or peptides per assay tube.

Discussion

The noncovalent reconstitution of a functional protein from its constituent inactive peptide fragments provides important information regarding the general principles that govern the folding of proteins to their unique three-dimensional structures (Anfinsen & Scheraga, 1975). Furthermore, it offers unique possibilities of examining the structure–function relationships of a given enzyme molecule. Before discussion in detail of the results obtained with thioredoxin-C′ in this paper, some unique features of thioredoxin should be pointed out. (1) Thioredoxin is a small protein with its functional groups contributed solely by amino acid side chains; it contains no metals or prosthetic groups (Holmgren, 1968) such as iron in ferredoxins or the heme in cytochrome *c*. This means that there is a direct relationship between the functions of residues in the stabilization of the three-dimensional structure and in catalytic functions. The functional group is a disulfide in a small loop (Holmgren et al., 1975) that is reversibly reduced to a dithiol with a concomitant local but largely unknown conformational change (Holmgren, 1972). Thioredoxin has thus two stable structures corresponding to the oxidized and the reduced form. During aerobic conditions only the oxidized form is stable. (2) The reaction of thioredoxin-S_2 with thioredoxin reductase and the reaction of thioredoxin-$(SH)_2$ with ribonucleotide reductase occur without any evidence for formation of ternary complexes with NADPH or CDP, respectively (Holmgren, 1980). Thus, both enzymes react with thioredoxin by ping-pong mechanisms. The apparent K_m values of thioredoxin for the two enzymes are in the range (2–4) × 10^{-6} M. In the enzymatic reactions, the protein–protein interactions thus take place with both reactants in the 10^{-6}–10^{-7} M concentration range. This implies critical binding interactions and complementarity in three-dimensional structures between thioredoxin and the two enzymes. Thus, the enzymatic activity assays are very sensitive in detecting the sum of any deviations from native thioredoxin due to either defective folded conformation, impaired binding, or destruction of catalytically important residues.

Structure of Thioredoxin-C′. The results of gel chromatography, gel electrophoresis, and activity measurements all show that thioredoxin-C′-S_2 is a relatively weak complex with a K_D in the range of 10^{-5}–10^{-6} M. Furthermore, the complex is in rapid reversible equilibrium with the free peptide fragments. The catalytic activity of thioredoxin-C′ as a substrate for thioredoxin reductase is high, whereas it is essentially inactive as a disulfide reductase with ribonucleotide reductase or insulin. These properties make it very different from thioredoxin-T′ (Slabý & Holmgren, 1979). A comparison of the two systems is shown in Table VIII. The conformations of the peptide fragments of the two complementing systems as they occur in thioredoxin-S_2 are shown in Figures 9 and 10. This kind of representation seems to be fully valid since the functional properties of the fragments that we can measure with enzymes or antibodies are based on the thioredoxin-S_2 structure. Furthermore, it is clear that reversible folding of peptide fragments into their "native format" takes place (Anfinsen & Scheraga, 1975). Fragments from a protein as rich in secondary structure as thioredoxin can a priori be expected to generate such folding, since α-helical and β-sheet regions of a peptide chain are thought to form nucleation centers for this process. The lower stability of thioredoxin-C′ may be caused by the nonconsecutive order of the β strands of the central core in thioredoxin (Figure 9). Thioredoxin-T′, on the other hand, is probably highly stable due to the possible self-folding properties of both peptide fragments. Once the fragments are in the complex, the hydrogen bonds between β_2 and β_4 provide the stabilization of the structure, and the long-range interactions providing the extra stability may be

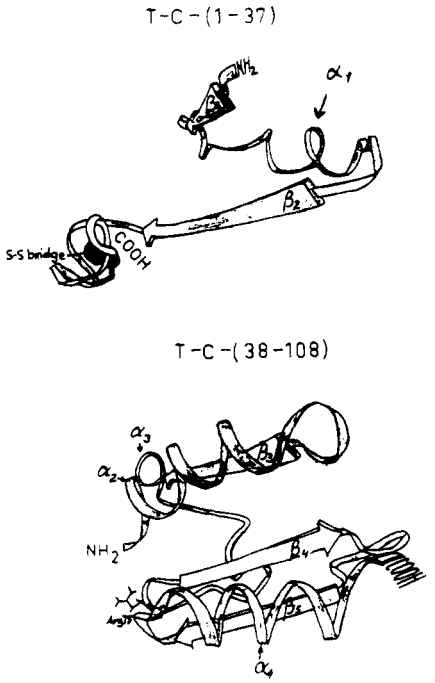

FIGURE 9: Thioredoxin-C-(1–37) and thioredoxin-C-(38–108) in their folded conformation as they occur in thioredoxin-S_2. This is from a drawing by Dr. Bo Furugren.

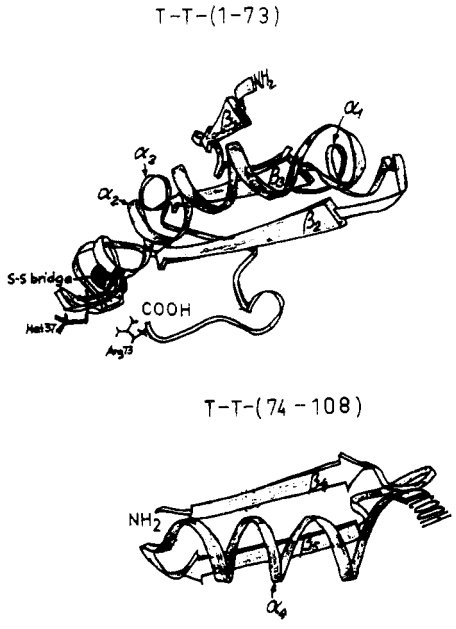

FIGURE 10: Thioredoxin-T-(1–73) and thioredoxin-T-(74–108) in their folded conformations as they occur in thioredoxin-S_2. This is from a drawing by Dr. Bo Furugren.

expressed. This is similar to the binding mode of subunits in a multimeric enzyme.

Why is thioredoxin-C′-$(SH)_2$ not active as a disulfide reductase? Probably because Met-37 is essential for the conformational change during the oxidoreduction of the disulfide in thioredoxin. This residue is conserved in thioredoxins from yeast (Hall et al., 1971) and mammalian liver.[2] Furthermore, the charge from the NH_2 group of Ile-38 and possibly the COOH group of the homoserine residue (Figure 9) will be expected to disturb the reactivity of the SH groups in thioredoxin. It is in fact more surprising that thioredoxin-C′-S_2

[2] A. Holmgren, unpublished results.

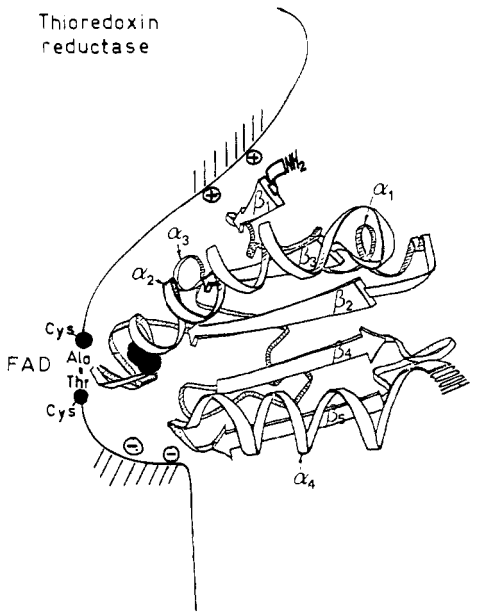

FIGURE 11: Proposed model for the interaction between *E. coli* thioredoxin-S_2 and *E. coli* thioredoxin reductase. Only one subunit of the enzyme is shown with FAD and the oxidation–reduction active disulfide (Thelander, 1970).

is highly active with thioredoxin reductase, despite these charges, close to the active disulfide.

Thioredoxin Reductase Reactions. Thioredoxin reductase from *E. coli* (M_r 70 000) contains two subunits, each with one residue of FAD and a redox-active disulfide bond. This disulfide is reduced to a dithiol by NADPH. The two-electron reduced enzyme then binds thioredoxin-S_2 and is thought to transfer the electrons to thioredoxin via thiol–disulfide interchange and an unstable mixed disulfide intermediate. The apparent dissociation constant for the thioredoxin-C′ complex and the K_m value of thioredoxin for thioredoxin reductase are of similar orders. The analysis of thioredoxin-C′-S_2 as a substrate for thioredoxin reductase is thus complicated by the dissociation of the complex and the inhibitory role of thioredoxin-C-(1–37). In our analysis we have assumed that the dithiol of thioredoxin-C′-$(SH)_2$ is rapidly reoxidized by DTNB.

The specificity of *E. coli* thioredoxin reductase for the disulfide of thioredoxin-S_2 is well documented [see Holmgren (1979)]. In fact, neither thioredoxin-C-(1–37) (Figure 9) nor thioredoxin-T-(1–73) (Figure 10) that contains the disulfide has any substrate activity even at high concentrations (Slaby & Holmgren, 1979). This may be because the binding interactions between thioredoxin-S_2 and thioredoxin reductase involve both the 1–37 region and the 74–108 region of the molecule. A hypothetical complex between thioredoxin-S_2 and thioredoxin reductase is presented in Figure 11. The model is based on results described in this and the preceding paper (Slaby & Holmgren, 1979) as follows. (1) Since thioredoxin-C-(1–37)-S_2 is an inhibitor of thioredoxin reductase under certain conditions, the model includes this region as an electrostatic binding site in thioredoxin-S_2. The negative charges may be a number of side chains (Asp-9, Asp-10, Asp-13, and Asp-15), which form a patch on the surface of thioredoxin.[3] Phage T4 thioredoxin is also a substrate for thioredoxin reductase (Berglund, 1969) but has a totally different primary structure when compared with *E. coli* thioredoxin (Sjöberg & Holmgren, 1972). Yet the three-

[3] B.-O. Söderberg, B.-M. Sjöberg, U. Sonnerstam, H. Eklund, C.-I. Brändén, and A. Holmgren, unpublished experiments.

dimensional structure of T4 thioredoxin is similar to that of *E. coli* thioredoxin (Söderberg et al., 1978), and a patch of negative charges has been observed in a corresponding position in the T4 molecule.[3]

(2) A region around Arg-73 in the 74–108 region is proposed to involve a second binding interaction. This is based on the low activity of thioredoxin-T′ (Slabý & Holmgren, 1979). Furthermore, a mutant thioredoxin (*E. coli* tsnC 7007), with the permissible Gly-92 exchanged to Asp-92,[4] has a dramatically changed reactivity with thioredoxin reductase.

(3) Lysine residues of the 1–37 sequence appear essential as seen from the chemical modification of thioredoxin-C-(1–37). Of special interest is Lys-36 which is a conserved residue[2] which may stabilize a thiolate ion base pair in thioredoxin-$(SH)_2$. In contrast, the folding of thioredoxin-C′ was possible from the lysine-modified thioredoxin-C-(1–37).

The model, in Figure 11, is a first attempt to describe the three-dimensional complementarity between thioredoxin and thioredoxin reductase. As can be seen, it leaves some areas of the thioredoxin surface free. In fact, when thioredoxin is bound to gene 5 as a subunit of phage T7 DNA polymerase (Mark & Richardson, 1976), it retains its activity with thioredoxin reductase.[4]

References

Ambler, R. P. (1965) *Biochem. J. 96*, 32P.
Anfinsen, C. B., & Scheraga, H. A. (1975) *Adv. Protein Chem. 29*, 205–300.
Berglund, O. (1969) *J. Biol. Chem. 244*, 6306–6308.
Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.
Dyckes, D. F., Creighton, T. E., & Sheppard, R. C. (1973) *Nature (London) 247*, 202–204.
Hall, D. E., Baldesten, A., Holmgren, A., & Reichard, P. (1971) *Eur. J. Biochem. 23*, 328–335.
Holmgren, A. (1968) *Eur. J. Biochem. 6*, 475–484.
Holmgren, A. (1972) *FEBS Lett. 24*, 351–354.
Holmgren, A. (1980) in *Dehydrogenases Requiring Nicotinamide Coenzymes* (Jeffrey, J., Ed.) Birkhäuser Verlag, Basel (in press).
Holmgren, A., & Reichard, P. (1967) *Eur. J. Biochem. 2*, 187–196.
Holmgren, A., & Sjöberg, B. M. (1972) *J. Biol. Chem. 247*, 4160–4164.
Holmgren, A., Söderberg, B. O., Eklund, H., & Brändén, C. I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2305–2309.
Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 317–330.
Light, A., Taniuchi, H., & Chen, R. F. (1974) *J. Biol. Chem. 249*, 2285–2293.
Mark, D., & Richardson, C. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 780–784.
Ornstein, L. (1964) *Ann. N.Y. Acad. Sci. 121*, 321–349.
Sjöberg, B.-M., & Holmgren, A. (1972) *J. Biol. Chem. 247*, 8063–8068.
Slabý, I., & Holmgren, A. (1975) *J. Biol. Chem. 250*, 1340–1347.
Slabý, I., & Holmgren, A. (1979) *Biochemistry* (preceding paper in this issue).
Söderberg, B.-O., Sjöberg, B.-M., Sonnerstam, U., & Brändén, C.-J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5827–5830.
Thelander, L. (1970) *J. Biol. Chem. 245*, 6026–6029.

[4] A. Holmgren, G.-B. Kallis, and B. Nordström, unpublished experiments.

Effect of an Induced Conformational Change on the Physical Properties of Two Chemotactic Receptor Molecules†

R. Suzanne Zukin,* P. R. Hartig, and D. E. Koshland, Jr.

ABSTRACT: The physical properties and conformational dynamics of the *Salmonella typhimurium* ribose and galactose receptors have been examined. Studies involving circular dichroism, fluorescence, absorption spectroscopy, and sedimentation analysis show that the two receptor proteins have different morphologies and exhibit diverse responses to sugar binding. The ribose receptor lacks both tryptophan and disulfide residues, and the galactose receptor lacks disulfides and has only a single tryptophan residue. By virtue of these fortuitous properties, the conformational changes induced in these proteins by sugar binding can be dissected by utilizing a variety of physical probes. A ligand-induced conformational change in the ribose receptor is shown by circular dichroism and fluorescence spectroscopy, which reveal spectral changes assignable to tyrosine, phenylalanine, and methionine residues. A conformational change in the galactose receptor has been demonstrated by fluorescence spectroscopy involving the distant reporter group method, which shows changes assignable to tryptophan and methionine sites and which is corroborated by sedimentation analysis. It is clear that there are extensive conformational changes in the two receptor proteins and that the different physical methods provide complementary information on the nature of these changes.

Physical probes have been widely used as tests of conformational change in protein molecules (Konev, 1967; Yu, 1977; James, 1978; Stryer, 1978). Upon ligand binding to a protein site, a signal associated with the probe may change in response either to direct interactions or to delocalized refolding of the macromolecule. Alternatively, physical properties of a reporter group may remain unchanged in reactions in which a ligand-induced conformational change does occur.

Physical probes of conformation change provide a dynamic picture of protein interactions not possible from X-ray crystallographic analysis. Such probes are of particular interest

† From the Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461 (R.S.Z.), and Department of Biochemistry, University of California, Berkeley, California 94720 (P.R.H. and D.E.K.). *Received May 14, 1979; revised manuscript received September 20, 1979.* Supported by National Science Foundation Grants BNS 78-17774 (to R.S.Z.) and PCM 76-21850 (to D.E.K) and U.S. Public Health Service Grant AM 09765 (to D.E.K).

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