Identification of Residues of Spinach Thioredoxin \( f \) That Influence Interactions with Target Enzymes

Mary K. Geck\(^{\dagger}\), Frank W. Larimer\(^{\dagger}\), and Fred C. Hartman\(^{\dagger}\)

From the \( ^{\dagger} \) Protein Engineering Program, Biology Division, Oak Ridge National Laboratory and the \( ^{\ddagger} \) University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee 37831

(Received for publication, July 2, 1996)

The necessity for two types of thioredoxins (Trx \( f \) and \( m \)) within chloroplasts of higher plants that mediate the same redox chemistry with various target enzymes is not well understood. To approach this complex issue, we have applied site-directed mutagenesis to the identification of residues of Trx \( f \) that affect its binding to and selectivity for target enzymes. Based upon amino acid sequence alignments and the three-dimensional structure of \( E. coli \) thioredoxin, putative key residues of Trx \( f \) were replaced with residues found at corresponding positions of Trx \( m \) to generate the mutants K58E, Q75D, N74D, and deletion mutants \( \Delta \) Asn-74 and \( \Delta \) Asn-77. Kinetics of activation of oxidized recombinant sorghum leaf NADP-dependent malate dehydrogenase and oxidized spinach chloroplastic fructose-1,6-bisphosphatase by wild-type Trx \( f \), wild-type Trx \( m \), and Trx \( f \) mutants were compared. All of the mutants are less efficient than wild-type Trx \( f \) in the activation of fructose-1,6-bisphosphatase and are altered in both \( S_{0.5} \) and \( V_{\text{max}} \). In contrast to literature reports, the activation of NADP-dependent malate dehydrogenase does not display rate saturation kinetics with respect to the concentration of Trx \( f \), thereby signifying very weak interactions between the two proteins. The mutants of Trx \( f \) likewise interact only weakly with NADP-dependent malate dehydrogenase, but the apparent second-order rate constants for activation are increased compared to that with wild-type Trx \( f \). Thus, Lys-58, Asn-74, Gln-75, and Asn-77 of Trx \( f \) contribute to its interaction with target enzymes and influence target protein selectivity.

Thioredoxins are ubiquitous, redox-active proteins that mediate thiol-disulfide exchanges with target enzymes (for reviews see Refs. 1–4). Two chloroplastic thioredoxins have been identified, designated \( f \) and \( m \), which can be reduced by ferredoxin as catalyzed by ferredoxin-thioredoxin reductase (for review, see Ref. 5). Both of these thioredoxins are crucial to the light regulation of \( \text{CO}_2 \) assimilation through the modulation of the redox status of various enzymes. Thioredoxins activate (or inactivate) target enzymes by a step-wise, thiol-disulfide exchange in which the enzyme disulfide undergoes nucleophilic attack by an active-site sulphydryl of the Trx \(^{\dagger} \) (Cys-46 in spinach Trx \( f \)) (6) to form a protein-protein mixed disulfide. The proximal active-site sulphydryl (Cys-49 in Trx \( f \)) then intramolecularly attacks the mixed disulfide, generating reduced enzyme and oxidized Trx. Trx \( f \) has been reported to selectively activate fructose-1,6-bisphosphatase (FBPase), sedoheptulose-1,7-bisphosphatase, phosphoribulokinase, and NADP-linked glyceraldehyde-3-phosphate dehydrogenase (1, 2). On the contrary, Trx \( m \) appears selective for NADP-dependent malate dehydrogenase (MDH) and chloroplastic glucose-6-phosphate dehydrogenase (7–9). A comparison of the amino acid sequences between the two thioredoxins shows only 29% identity but complete conservation of the active-site sequence Cys-Gly-Pro-Cys (10).

The basis for the existence of two types of thioredoxins within the chloroplasts of higher plants that preferentially mediate the same redox chemistry of different target enzymes is unknown. As one approach to this issue, we are exploring, by site-directed mutagenesis, the roles of residues that appear unique to Trx \( f \) relative to other thioredoxins. One such residue is Lys-58, which is replaced by glutamate in spinach Trx \( m \), \( E. coli \) Trx, and indeed the majority of thioredoxins in other organisms (Fig. 1) (10). Based on the structure of \( E. coli \) Trx (11–13), Lys-58 is positioned on the surface, is salt-bridged with residue 110 (Asp in Trx \( f \); Lys in \( E. coli \) Trx and Trx \( m \)), and is located near the active site. Hence, we were prompted to construct and characterize the K58E mutant of Trx \( f \) as reported herein.

Residues 74, 75, and 77 were also examined. These residues are located near a conserved, putative hydrophobic contact surface encompassing Gly-33, Pro-34, Ile-75, Pro-76, Val-91, and Asn-77 of Trx \( f \) contribute to its interaction with target enzymes and influence target protein selectivity.

\(^{\dagger}\) This work was supported by Grant 94–37396–0337 from the National Research Initiative Competitive Grants Program of the United States Department of Agriculture and by the Office of Health and Environmental Research, United States Department of Energy under Contract DE-AC0596OR22464 with Lockheed Martin Energy Systems, Inc. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\(^{\ddagger}\) To whom correspondence should be addressed: Biology Division, Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8077. Tel.: 423-574-0212; Fax: 423-574-9297; E-mail: hartmanfc@ornl.gov.

\(^{1}\) The abbreviations used are: Trx, thioredoxin; FBP, fructose 1,6-bisphosphate; FBPase, fructose-1,6-bisphosphatase; MDH, NADP-dependent malate dehydrogenase.
Interaction of Thioredoxin f with Target Enzymes

EXPERIMENTAL PROCEDURES

Materials—Oligonucleotides were synthesized by phosphoramidite chemistry with an Applied Biosystems model 392 DNA/RNA synthesizer. 5'-[α-32P]dATP (1000 Ci/mmol) was obtained from DuPont NEN. Bacto-tryptone and bacto-yeast extract were obtained from Difco. Di-thiothreitol, N,N'-bis-(2-hydroxyethyl)glycine, HEPES, and Tris (free base) were purchased from Research Organics. Assay coupling enzymes, NAD, NADPH, oxaloacetate, EDTA, and bovine serum albumin were from Sigma. FBP was from Boehringer Mannheim. [a-35S]dATP (1000 Ci/mmol) was obtained from DuPont NEN. Assay solutions (1 ml) consisted of 50 mM HEPES-KOH (pH 7.8), 50 μM EDTA, and 5 mM dithiothreitol. The formation of NADH was followed spectrophotometrically at 340 nm. The rates reported are corrected for the absorbance change of 6.22/min. The rates reported are corrected for the absorbance change of 6.22/min.

Activation of FBPase by Trx—Rates of activation of oxidized FBPase (40 μM) as a function of Trx concentration were determined at 25°C in 50 mM HEPES-KOH (pH 7.8), 0.1 mM EDTA, and 5 mM dithiothreitol. Periodically, 40-μl aliquots of the reaction mixture were removed and assayed for FBPase activity. Assay solutions (1 ml) consisted of 50 mM HEPES-KOH (pH 7.8), 50 μM EDTA, 1 mM MgSO4, 4 mM FBP, 2 units of phosphoglucose isomerase, 3 units of glucose-6-phosphate dehydrogenase, and 1 mM NADP-. The formation of NADH was followed spectrophotometrically at 340 nm. One unit of FBPase activity is represented by an absorbance change of 6.22/min.

Activation of MDH by Trx—Rates of activation of oxidized MDH (2.6 μM) as a function of Trx concentration were determined at 4°C in 100 mM Tris-HCl (pH 7.9), 10 mM dithiothreitol, 1 mM EDTA, and 1 mg/ml bovine serum albumin. At various times, 20-μl aliquots of the reaction mixture were removed and assayed for MDH activity. The assays, in a 100-μl volume, were performed at 25°C in 100 mM Tris-HCl (pH 7.9), 1 mM EDTA, 1 mg/ml bovine serum albumin, 1.5 mM oxaloacetate, and 0.3 mM NADPH. The oxidation of NADPH was followed spectrophotometrically at 340 nm. One unit of MDH activity corresponds to an absorbance change of 0.02/minute.

RESULTS

Published values for kinetic parameters of activation of FBPase and MDH by Trx f or Trx m vary widely. Thus, direct determination of the quantitative impact of the designed structural alterations of Trx f required a re-examination of the

FIG. 1. Alternate sequence alignments of thioredoxins. Conserved active site sequence and sites of mutation in Trx f are indicated in bold.

| A | Spinach f | MEQALGQXME | V | G | K | V | T | E | V | N | K | D | T | F | P | W | F | P | V | A | G | D | K | X | D | W | F | T | Q |
| B | Spinach m | MeQAXME | V | K | E | V | Q | D | V | N | D | S | S | K | E | F | V | L | E | S | K | P | V | M | D | F | W | A | P |

The formation of NADH was followed spectrophotometrically at 340 nm.

Interaction of Thioredoxin f with Target Enzymes

EXPERIMENTAL PROCEDURES

Materials—Oligonucleotides were synthesized by phosphoramidite chemistry with an Applied Biosystems model 392 DNA/RNA synthesizer. 5'-[α-32P]dATP (1000 Ci/mmol) was obtained from DuPont NEN. Bacto-tryptone and bacto-yeast extract were obtained from Difco. Di-thiothreitol, N,N'-bis-(2-hydroxyethyl)glycine, HEPES, and Tris (free base) were purchased from Research Organics. Assay coupling enzymes, NAD, NADPH, oxaloacetate, EDTA, and bovine serum albumin were from Sigma. FBP was from Boehringer Mannheim. [a-35S]dATP (1000 Ci/mmol) was obtained from DuPont NEN. Assay solutions (1 ml) consisted of 50 mM HEPES-KOH (pH 7.8), 50 μM EDTA, and 5 mM dithiothreitol. The formation of NADH was followed spectrophotometrically at 340 nm. The rates reported are corrected for the absorbance change of 6.22/min. The rates reported are corrected for the absorbance change of 6.22/min.

Activation of FBPase by Trx—Rates of activation of oxidized FBPase (40 μM) as a function of Trx concentration were determined at 25°C in 50 mM HEPES-KOH (pH 7.8), 0.1 mM EDTA, and 5 mM dithiothreitol. Periodically, 40-μl aliquots of the reaction mixture were removed and assayed for FBPase activity. Assay solutions (1 ml) consisted of 50 mM HEPES-KOH (pH 7.8), 50 μM EDTA, 1 mM MgSO4, 4 mM FBP, 2 units of phosphoglucose isomerase, 3 units of glucose-6-phosphate dehydrogenase, and 1 mM NADP-. The formation of NADH was followed spectrophotometrically at 340 nm. One unit of FBPase activity is represented by an absorbance change of 6.22/min.

Activation of MDH by Trx—Rates of activation of oxidized MDH (2.6 μM) as a function of Trx concentration were determined at 4°C in 100 mM Tris-HCl (pH 7.9), 10 mM dithiothreitol, 1 mM EDTA, and 1 mg/ml bovine serum albumin. At various times, 20-μl aliquots of the reaction mixture were removed and assayed for MDH activity. The assays, in a 100-μl volume, were performed at 25°C in 100 mM Tris-HCl (pH 7.9), 1 mM EDTA, 1 mg/ml bovine serum albumin, 1.5 mM oxaloacetate, and 0.3 mM NADPH. The oxidation of NADPH was followed spectrophotometrically at 340 nm. One unit of MDH activity corresponds to an absorbance change of 0.02/minute.

RESULTS

Published values for kinetic parameters of activation of FBPase and MDH by Trx f or Trx m vary widely. Thus, direct determination of the quantitative impact of the designed structural alterations of Trx f required a re-examination of the
properties of the wild-type thioredoxins. Consistent with prior reports (9, 23), wild-type Trx f is far superior to wild-type Trx m in the activation of FBPase, displaying a 35-fold higher affinity and a 25-fold increased $V_{\text{max}}$ (Fig. 3A and Table I). All of the mutants analyzed are impaired relative to wild-type Trx f but are still superior to Trx m when analyzed with FBPase (Fig. 3B and Table I). The largest deviation from the $S_{0.5}$ value of 0.9 $\mu$m determined with wild-type Trx f occurs with the Asn-77 deletion mutant, in which case the affinity is lowered about 15-fold. Among the mutants analyzed, replacement of Lys-58 by a glutamyl residue has the largest impact on $V_{\text{max}}$ with a decrease of 3-fold.

As anticipated, Trx m is far more effective as an activator for MDH than for FBPase. Indeed, the rates of activation of MDH by Trx m are so rapid that reliable monitoring could only be achieved at 4°C as opposed to room temperature. Even without correction for this differential in temperature, the $V_{\text{max}}$ for Trx m in the activation of MDH is 21 mU/min (Fig. 4) compared to 2 mU/min in the activation of FBPase (Fig. 3A and Table I); however, the $S_{0.5}$ values for the interaction of Trx m with the two enzymes are both approximately 30 $\mu$m. Despite the efficiency of activation of MDH by Trx m, a comparison of the activation profiles for MDH by Trx f and Trx m argues against preferential regulation by the latter (Fig. 4). Even though rate saturation with respect to Trx f concentrations up to 50 $\mu$m cannot be demonstrated, signifying very weak association between Trx f and MDH, the rates of activation of MDH by Trx f are more rapid than those with Trx m throughout the concentration range examined.

The activation kinetics of MDH by the mutants of Trx f are complex, exhibiting pronounced sigmoidicity as seen with wild-type Trx f (Fig. 5). However, analogous to wild-type Trx f, the mutants do not show rate saturation in activation of MDH. Thus, the relative effectiveness of Trx f mutants as activators of MDH are expressed as apparent second-order rate constants (Table I) calculated from the linear region of the activation curves (Fig. 5). Based on this criterion, all mutants are more effective than wild-type Trx f in the activation of MDH.

**DISCUSSION**

Despite both qualitative and quantitative variations among published data, several laboratories have confirmed differential selectivities of Trx f and Trx m for target enzymes as discovered in pioneering studies by Buchanan and colleagues (3, 9). As the redox potentials of these two thioredoxins are identical (~210 mV) (24), their selectivities must reflect differential affinities for target enzymes and/or differential propensities for sulphydryl-disulfide exchanges within the Trx-enzyme complexes. The present study was predicated on the supposition that recognition and selectivity determinants of Trx f and Trx m could be determined by site-directed mutagenesis as guided by clearly discernible structural differences between the two thioredoxins. Satisfyingly, the residue (Lys-58) and segment (positions 74–77) of Trx f chosen for change are shown to influence its efficiency ($V_{\text{max}}/S_{0.5}$) and selectivity in the activa-
Interaction of Thioredoxin f with Target Enzymes

The impaired efficiency of K58E, relative to wild-type Trx f, in the activation of FBPase is due primarily to a lower $V_{\text{max}}$, indicative of a slower rate of thiol-disulfide exchange within the productive complex. Slower exchange could reflect misalignment of the exchanging groups or altered pK$_a$ values of the active-site sulfydryls of Trx f. Certainly, the functional group of residue 58 is too far removed, 15–20 Å as extrapolated from the three-dimensional structures of E. coli Trx (11, 12), human Trx (25), and Anabaena Trx-2 (26), from the active-site sulfydryls to directly influence their reactivities. However, either direct line.

tion of target enzymes.

The impaired efficiency of K58E, relative to wild-type Trx f, in the activation of FBPase is due primarily to a lower $V_{\text{max}}$, indicative of a slower rate of thiol-disulfide exchange within the productive complex. Slower exchange could reflect misalignment of the exchanging groups or altered pK$_a$ values of the active-site sulfydryls of Trx f. Certainly, the functional group of residue 58 is too far removed, 15–20 Å as extrapolated from the three-dimensional structures of E. coli Trx (11, 12), human Trx (25), and Anabaena Trx-2 (26), from the active-site sulfydryls to directly influence their reactivities. However, either direct line.

The efficiency of activation of FBPase by Trx f is very sensitive to changes in residues at positions 74–77 with greater than 9-fold declines among three of the five mutants examined. In contrast to K58E, these mutants are less efficient than wild-type Trx f due primarily to weakened interactions with FBPase as gauged by $S_{0.5}$ values. Therefore, we conclude that residues 74–77 serve as an important recognition site for target enzymes. The earlier finding that replacement of Asp-61 of E. coli Trx with Asn, as located at the corresponding position 74 in Trx f improves the activation parameters with FBPase (16), is consistent with this conclusion. Our conclusion is further supported by structural information derived from both human and Anabaena Trx. With human Trx, long range nuclear Over-

![FIG. 4. Activation of MDH by wild-type thioredoxins.](image)

![FIG. 5. Activation of MDH by mutant thioredoxins.](image)

**TABLE II**

| Thioredoxin      | Rate constant$^a$ (min$^{-1}$) |
|------------------|-------------------------------|
| Wild-type Trx f  | 1.2 × 10$^4$                  |
| Wild-type Trx m  | 9.8 × 10$^3$                  |
| Trx fK58E        | 3.8 × 10$^3$                  |
| Trx fΔN74        | 3.7 × 10$^4$                  |
| Trx fN74D        | 1.5 × 10$^4$                  |
| Trx fQ75D        | 2.8 × 10$^4$                  |
| Trx fΔN77        | 2.4 × 10$^4$                  |

$^a$ The value given for wild-type Trx f is a true second-order rate constant, reflective of first-order activation kinetics with respect to the concentration of Trx f. All other values denote apparent second-order rate constants calculated from activation rates at 40 μM Trx.

Whereas all of the engineered structural changes of Trx f were counterproductive with respect to activation of FBPase, they were beneficial with respect to activation of MDH, thereby validating the conversion of Trx f to a more “m-like” thioredoxin. Although $V_{\text{max}}$ and $S_{0.5}$ values for the activation of MDH by the Trx mutants could not be determined due to the absence of rate saturation even at 1000-fold molar excess of the given thioredoxin, the observed activation rates were greater with the mutants than with wild-type Trx f at concentrations exceeding 30 μM. The Trx f mutants were also more akin to Trx m with respect to kinetics of MDH activations, displaying sigmoidicity as opposed to linearity with wild-type Trx f.

In addition to identifying functional and selectivity elements of Trx f, the present study partially addresses some of the published conflicts about relative target enzyme selectivities of Trx f and Trx m. Although all accounts agree that Trx f is superior to Trx m in the activation of FBPase (9, 23, 29), some allege that this enzyme is totally refractive to Trx m (30, 31). Given the inefficiency of Trx m in the activation of FBPase, 550-fold lower than that of Trx f, we surmise that negative reports merely reflect insufficient concentrations and reaction times.

Beyond these readily reconcilable differences, reports concerning activation of MDH range from a striking preferential effectiveness of Trx m (9, 32) to superiority of Trx f (30, 33) as we observe. These disparities could be due to differences in conditions for activation or differences in approaches to determining kinetic parameters. Shortly after the discovery of Trx f and Trx m (9), preferential activation of MDH by the latter was reported to be dependent on the presence of high concentrations of chloride (32). Although we observe stimulation of thioredoxin-dependent activation of MDH by 250 mM NaCl, the
Interaction of Thioredoxin f with Target Enzymes

impact is similar for both Trx \( m \) and Trx \( f \). We thus believe that methods, rather than conditions, are the more likely basis of variable results among different laboratories. In most prior publications, the level of activation at a given Trx concentration is based on a single, fixed time point assay; the rate of activation is then assumed to be linear throughout the time period selected. Thus, to some extent, equilibria are being determined rather than rates at which equilibria are attained. The kinetic patterns presently reported are based on true initial rates of activation, whereby an incubation mixture of Trx and target enzyme is sampled repeatedly in order to reveal the linear phase of time-dependent activation. Prior oversights to clearly distinguish rates and equilibria can readily account for the wide range of apparent affinities of spinach Trx \( f \) for spinach FBPase; e.g. \( S_{0.5} \) values of 1.6 nM (23), 0.19 \( \mu \)M (34), and 1 \( \mu \)M (6, 16) have been reported. Given this enormous variation of apparent affinities with one enzyme system, reports of inverse selectivities of Trx \( f \) and Trx \( m \) are understandable. Although two prior studies include true rates of activation of Trx-regulated enzymes, quantitative comparisons with our data are precluded. One report was restricted to an examination of the Trx \( m/MDH \) system and did not entail a complete profile of the rate dependence on Trx \( m \) concentration (35). In the other report, the activation of FBPase by Trx \( f \) was examined at molar ratios that gave rise to first-order kinetics with respect to Trx \( f \) concentration (i.e. absence of rate saturation) (36).

Based on our finding of clear-cut kinetic superiority of Trx \( f \) relative to Trx \( m \) in the \textit{in vitro} activation of MDH, we suggest that the physiological role of Trx \( m \) should be reassessed.

Acknowledgments—We are extremely grateful to Professor Peter Schurrmann of the Université de Neuchâtel, Switzerland and Professor Jean-Pierre Jacquot of the Université Paris-Sud, France for their gifts of Trx \( m \) and MDH, respectively.

REFERENCES

1. Holmgren, A., Branden, C.-I., Jorvall, H., and Sjöberg, B.-M. (eds) (1986) \textit{Thioredoxin and Glutaredoxin Systems: Structure and Function}, Raven Press, NY
2. Buchanan, B. B. (1991) \textit{Arch. Biochem. Biophys.} \textbf{285}, 1–9
3. Buchanan, B. B. (1980) \textit{Annu. Rev. Plant Physiol. Plant Mol. Biol.} \textbf{31}, 341–374
4. Buchanan, B. B. (1992) in \textit{Trends in Photosynthesis Research} (Barber, J., Guerrero, M. G., and Medrano, H., eds) pp. 171–183, Intercept Ltd., Andover, UK
5. Buchanan, B. B., Schurrmann, P., Decottignies, P., and Lazzaro, R. M. (1994) \textit{Arch. Biochem. Biophys.} \textbf{314}, 257–260
6. Brandes, H. K., Larimer, F. W., Geck, M. K., Stringer, C. D., Schurrmann, P., and Hartman, F. C. (1993) \textit{J. Biol. Chem.} \textbf{268}, 18411–18414
7. Jacquot, J.-P., Vidal, J., Gadal, P., and Schurrmann, P. (1978) \textit{FEBS Lett.} \textbf{96}, 243–246
8. Scheibe, R., and Anderson, L. E. (1981) \textit{Biochim. Biophys. Acta} \textbf{636}, 58–64
9. Wolosiuk, R. A., Crawford, N. A., Yee, B. C., Buchanan, B. B. (1979) \textit{J. Biol. Chem.} \textbf{254}, 1627–1632
10. Eklund, H., Gleason, F. K., and Holmgren, A. (1991) \textit{Proteins Struct. Funct. Genet.} \textbf{11}, 13–28
11. Dyson, H. J., Gippert, G. P., Case, D. A., Holmgren, A., and Wright, P. E. (1990) \textit{Biochemistry} \textbf{29}, 4129–4136
12. Katti, S. K., LeMaster, D. M., and Eklund, H. (1990) \textit{J. Mol. Biol.} \textbf{212}, 167–184
13. Jung, M. F., Campbell, A. P., Begley, T., Holmgren, A., Case, D. A., Wright, P. E., and Dyson, H. J. (1994) \textit{Structure} \textbf{2}, 855–868
14. Xia, T.-H., Bushweller, J. H., Sodano, P., Billeter, M., Bjernberg, O., Holmgren, A., and Wuthrich, K. (1992) \textit{Protein Sci.} \textbf{1}, 310–321
15. Holmgren, A. (1985) \textit{Annu. Rev. Biochem.} \textbf{54}, 237–271
16. de Lamotte-Gueray, F., Migniac-Maslow, M., Decottignies, P., Stein, M., Minard, P., and Jacquot, J.-P. (1991) \textit{Eur. J. Biochem.} \textbf{196}, 287–294
17. Wodel, N., Clausmeyer, S., Hermann, R. G., Gardet-Salvi, L., and Schurrmann, P. (1992) \textit{Plant Mol. Biol.} \textbf{18}, 527–533
18. Kunkel, T. A., Roberts, J. D., Zakour, R. A. (1987) \textit{Methods Enzymol.} \textbf{154}, 367–382
19. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{74}, 5463–5467
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) \textit{Molecular Cloning: A Laboratory Manual}, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Zimmermann, G., Kelly, G. J., and Latzko, E. (1976) \textit{Eur. J. Biochem.} \textbf{70}, 263–267
22. Marcus, F., Harroch, P. B., Moberly, L., Edelstein, I., and Latshaw, P. S. (1987) \textit{Biochemistry} \textbf{26}, 7029–7035
23. Haberlein, L., and Vogeler, B. (1995) \textit{Biochim. Biophys. Acta} \textbf{1253}, 169–174
24. Sambrook, J., Tolkan, G., Hirasawa, M., Gardet-Salvi, L., Stritt-Erter, A.-L., Knaff, D. B., and Schurrmann, P. (1995) \textit{Biochim. Biophys. Acta} \textbf{1230}, 114–118
25. Qin, J., Clore, G. M., and Gronenborn, A. M. (1994) \textit{Structure} \textbf{2}, 503–522
26. Saarinen, M., Gleason, F. K., and Eklund, H. (1995) \textit{Structure} \textbf{3}, 1097–1108
27. Qin, J., Clore, G. M., Kennedy W. M. P., Huth, J. R., and Gronenborn, A. M. (1995) \textit{Structure} \textbf{3}, 289–297
28. Gleason, F. K. (1992) \textit{J. Bacteriol.} \textbf{174}, 2592–2598
29. Nishizawa, A. N., and Buchanan, B. B. (1981) \textit{J. Biol. Chem.} \textbf{256}, 6119–6126
30. Schurrmann, P., Maeda, K., and Tsugita, A. (1981) \textit{Eur. J. Biochem.} \textbf{116}, 37–45
31. Tsugita, A., Maeda, K., and Schurrmann, P. (1983) \textit{Biochim. Biophys. Res. Commun.} \textbf{115}, 1–7
32. Crawford, N. A., Yee, B. C., Hutcheson, S. W., Wolosiuk, R. A., and Buchanan, B. B. (1986) \textit{Arch. Biochem. Biophys.} \textbf{244}, 1–15
33. Hodges, M., Migniac-Maslow, M., Decottignies, P., Jacquot, J.-P., Stein, M., Lepiniec, L., Cretin, C., and Gadal, P. (1994) \textit{Plant Mol. Biol.} \textbf{22}, 225–234
34. Aguilar, F., Brunnner, B., Gardet-Salvi, L., Stutz, E., and Schurrmann, P. (1992) \textit{Plant Mol. Biol.} \textbf{20}, 301–306
35. Scheibe, R., Fickensher, K., and Ashton, A. R. (1986) \textit{Biochim. Biophys. Acta} \textbf{870}, 191–197
36. Clancey, C. J., and Gilbert, H. F. (1987) \textit{J. Biol. Chem.} \textbf{262}, 13545–13549