Thioredoxin from *Rhodospirillum rubrum*: Primary Structure and Relation to Thioredoxins from Other Photosynthetic Bacteria

THOMAS C. JOHNSON,¹ BOIHON C. YEE,¹ DONALD E. CARLSON,¹ BOB B. BUCHANAN,^{1*}
RICHARD S. JOHNSON,² W. RODNEY MATHEWS,^{2†} AND KLAUS BIEMANN²

Division of Molecular Plant Biology, University of California, Berkeley, California 94720,¹ and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139²

Received 3 November 1987/Accepted 2 February 1988

Thioredoxin was isolated from a photosynthetic purple nonsulfur bacterium, *Rhodospirillum rubrum*, and its primary structure was determined by high-performance tandem mass spectrometry. The sequence identity of *R. rubrum* thioredoxin to *Escherichia coli* thioredoxin was intermediate to those of the *Chlorobium thiosulfatophilum* and *Chromatium vinosum* proteins. The results indicate that *R. rubrum* has an NADP-thioredoxin system similar to that of other photosynthetic purple bacteria.

Thioredoxins are proteins, typically with molecular weights of 12,000, that are widely distributed in the animal, plant, and microbial kingdoms (7). All authentic thioredoxins examined show the same active site (Trp-Cys-Gly-Pro-Cys) in which they undergo reversible reduction and oxidation of the disulfide (S-S $\rightleftharpoons$ 2SH). Depending on the organism, thioredoxins can be reduced enzymatically with either reduced ferredoxin (in oxygenic photosynthesis and fermentation) or NADPH₂ (in anoxygenic photosynthesis and heterotrophy) (1, 4). Thioredoxins appear to play a central role in the regulation of enzymes in oxygenic photosynthesis, but their role in anoxygenic photosynthetic and heterotrophic organisms is not clear (7).

Thioredoxins have been purified from photosynthetic bacteria (3, 10), and in two cases, primary structures have been determined by a new method employing fast-atom-bombardment tandem mass spectrometry alone (9) or combined with Edman degradation (12). The two thioredoxins that have been sequenced are definitely related, but they show significant structural differences. For example, the thioredoxin from the purple sulfur bacterium *Chromatium vinosum* is more similar to its *Escherichia coli* counterpart than to thioredoxin from the green sulfur bacterium *Chlorobium thiosulfatophilum*.

In view of the structural differences in the thioredoxins from the two photosynthetic bacteria studied thus far, it is of interest to examine the amino acid sequence and related properties of thioredoxin from a third major group of these organisms, viz., the purple nonsulfur bacteria. We have, therefore, utilized the new methodology (9) to approach this question and now report that, in relation to *E. coli*, the sequence identity of *Rhodospirillum rubrum* thioredoxin is intermediate between those of its *C. thiosulfatophilum* and *Chromatium vinosum* counterparts. Certain other features of the *R. rubrum* thioredoxin system are also described.

R. rubrum S-I thioredoxin was isolated from cells grown photoheterotrophically on malate (2) by the procedure described for *C. thiosulfatophilum* (12). Thioredoxin was assayed by measuring its ability to activate NADP-malate dehydrogenase from corn chloroplasts in the presence of dithiothreitol (10). NADP-thioredoxin reductase (NTR) was assayed by following the reduction of 5,5'-dithiobis(2-nitro-

benzoic acid) in the presence of thioredoxin (10). The primary structure for the thioredoxin isolated from *R. rubrum* was determined by high-performance tandem mass spectrometry as described earlier for the related protein from *Chromatium vinosum* (9). Differentiation of leucine and isoleucine was based on ions resulting from β,γ -bond cleavage, if such ions were present (9a). Biochemicals were purchased from Sigma Chemical Co., St. Louis, Mo., except for *E. coli* NTR which was purchased from IMCO Corp., Stockholm, Sweden.

Purification and characterization. The purification procedure developed with other photosynthetic bacteria (12) was successfully applied for the isolation of thioredoxin from *R. rubrum* (Table 1). The procedure, carried out with photoheterotrophically grown cells, gave evidence for one thioredoxin which was active in the dithiothreitol-dependent activation of corn NADP-malate dehydrogenase (the assay routinely used to monitor activity), as well as in the NADPH-dependent reduction of 5,5'-dithiobis(2-nitrobenzoic acid) measured in the presence of *E. coli* NTR. *R. rubrum* thioredoxin showed no activity in the 5,5'-dithiobis(2-nitrobenzoic acid) reduction assay with the NTR recently identified and purified from spinach leaves (F. J. Florencio, B. C. Yee, T. C. Johnson, and B. B. Buchanan, unpublished results). Significantly, *R. rubrum* preparations showed an NTR activity that was separated from thioredoxin on DEAE (DE52)-cellulose or gel filtration columns (10). The data thus indicate that *R. rubrum* contained an NADP-thioredoxin system analogous to that of other purple bacteria, i.e., *Chromatium vinosum* (10) and *Rhodobacter sphaeroides* (3).

Structural analysis. To determine the primary structure of *R. rubrum* thioredoxin, four sets of proteolytic peptides were examined: (i) trypsin-digested peptides, (ii) *Staphylococcus aureus* (V8) protease-digested tryptic peptides isolated by reversed-phase high-pressure liquid chromatography, (iii) *S. aureus* protease-digested peptides, and (iv) chymotrypsin-digested selected *S. aureus* protease peptides (Fig. 1). To confirm independently the finding that the N terminus appeared to be shortened relative to the *E. coli* thioredoxin sequence (see Fig. 2), the intact protein was also subjected to 10 cycles of Edman sequencing. The results agreed with the sequence deduced from the mass spectral data.

R. rubrum thioredoxin was also analyzed by a newly

* Corresponding author.

† Present address: The Upjohn Company, Kalamazoo, MI 49001.

TABLE 1. Purification of *R. rubrum* thioredoxin

Step	Total activity ^a	Sp act ^b	% Recovery
Sonic extract	ND ^c	ND	ND
Acid (pH 4.7) supernatant	ND	ND	ND
Acetone	130	0.07	100
DE52 chromatography	92	0.42	71
Sephadex G-75 filtration	56	14.7	43
Mono Q chromatography	41	20.8	32 ^d

^a Micromoles of NADPH oxidized per minute.^b Micromoles of NADPH oxidized per minute per milligram of protein.^c ND, No data on which to base calculation.^d Corresponds to about 2 mg of thioredoxin from 230 g of cell paste.

devised fragmentation process allowing the differentiation of leucine and isoleucine (9a). Each leucine or isoleucine was differentiated in this manner except for those at positions 21 and 35. When considered in the light of this fragmentation process, further interpretation of the tandem mass spectra of the proteolytic peptides from the thioredoxins of *Chromatium vinosum* and *C. thiosulfatophilum* made it possible to differentiate most of their leucines and isoleucines so that only four and five positions, respectively, now remain undifferentiated (see Fig. 2).

The amino acid sequence of *R. rubrum* thioredoxin is shown in Fig. 2, along with those of the photosynthetic bacteria *Chromatium vinosum* and *C. thiosulfatophilum* and the heterotrophic bacterium *E. coli*. While the total number of amino acids present varied slightly, there were several major sites of strictly conserved homology among the four thioredoxins (with *E. coli* thioredoxin as the reference): (i) the region preceding the active site (residues 25, 26, 28, and 29), (ii) the active site (residues 31 to 35), (iii) and two regions following the active site (residues 56, 57, 59, 61, 63, and 64 and residues 70, 71, 73, and 75 to 77). In addition, there were several other points of less extensive conservation (residues 9, 12, 40, 46, 84, 86, 92, 93, and 96).

<i>E. coli</i>	Ser	Asp	Lys	Ile	Ile	His	Leu	Thr	Asp	Asp	Ser	Phe	Asp	Thr	Asp	15
<i>Chromatium</i>	Ser	Asp	Ser	Ile	Val	His	Val	Thr	Asp	Asp	Ser	Phe	Glu	Glu	Glu	
<i>Chlorobium</i>	Ala	Gly	Lys	Tyr	Phe	Glu	Ala	Thr	Asp	Lys	Asn	Phe	Gln	Thr	Glu	
<i>Rhodospirillum</i>				Met	Lys	Gln	Val	Ser	Asp	Ala	Ser	Phe	Glu	Glu	Asp	
<i>E. coli</i>	Val	Leu	Lys	Ala	Asp	Gly	Ala	Ile	Leu	Val	Asp	Phe	Trp	Ala	Glu	30
<i>Chromatium</i>	Val	Xle	Lys	Ser	Pro	Asp	Pro	Val	Leu	Val	Asp	Tyr	Trp	Ala	Asp	
<i>Chlorobium</i>	Ile	Leu	Asp	Ser	Asp	Lys	Ala	Val	Xle	Val	Asp	Phe	Trp	Ala	Ser	
<i>Rhodospirillum</i>	Val	Leu	Lys	Ala	Asp	Gly	Pro	Val	Xle	Val	Asp	Phe	Trp	Ala	Glu	
<i>E. coli</i>	Trp	Cys	Gly	Pro	Cys	Lys	Met	Ile	Ala	Pro	Ile	Leu	Asp	Glu	Ile	45
<i>Chromatium</i>	Trp	Cys	Gly	Pro	Cys	Lys	Met	Ile	Ala	Pro	Val	Leu	Asp	Glu	Ile	
<i>Chlorobium</i>	Trp	Cys	Gly	Pro	Cys	Met	Met	Xle	Gly	Pro	Val	Ile	Asp	Glu	Leu	
<i>Rhodospirillum</i>	Trp	Cys	Gly	Pro	Cys	Arg	Gln	Xle	Ala	Pro	Ala	Leu	Glu	Glu	Leu	
<i>E. coli</i>	Ala	Asp	Glu	Tyr	Gln	Gly	Lys	Leu	Thr	Val	Ala	Lys	Leu	Asn	Ile	60
<i>Chromatium</i>	Ala	Asp	Glu	Tyr	Ala	Gly	Arg	Val	Xle	Ala	Lys	Xle	Asn	Ile		
<i>Chlorobium</i>	Ala	Asp	Asp	Tyr	Glu	Gly	Lys	Ala	Ile	Ile	Ala	Lys	Xle	Asn	Val	
<i>Rhodospirillum</i>	Ala	Thr	Ala	Leu	Gly	Asp	Lys	Val	Thr	Val	Ala	Lys	Ile	Asn	Ile	
<i>E. coli</i>	Asp	Gln	Asn	Pro	Gly	Thr	Ala	Pro	Lys	Tyr	Gly	Ile	Arg	Gly	Ile	75
<i>Chromatium</i>	Asp	Glu	Asn	Pro	Asn	Thr	Pro	Pro	Arg	Tyr	Gly	Ile	Arg	Gly	Ile	
<i>Chlorobium</i>	Asp	Glu	Asn	Pro	Asn	Ile	Ala	Gly	Gln	Tyr	Gly	Xle	Arg	Ser	Ile	
<i>Rhodospirillum</i>	Asp	Glu	Asn	Pro	Gln	Thr	Pro	Ser	Lys	Tyr	Gly	Val	Arg	Gly	Ile	
<i>E. coli</i>	Pro	Thr	Leu	Leu	Leu	Phe	Lys	Asn	Gly	Glu	Val	Ala	Ala	Thr	Lys	90
<i>Chromatium</i>	Pro	Thr	Leu	Met	Leu	Phe	Arg	Gly	Gly	Glu	Val	Glu	Ala	Thr	Lys	
<i>Chlorobium</i>	Pro	Thr	Met	Leu	Ile	Xle	Lys	Gly	Gly	Lys	Val	Val	Asp	Gln	Met	
<i>Rhodospirillum</i>	Pro	Thr	Leu	Met	Ile	Phe	Lys	Asp	Gly	Gln	Val	Ala	Ala	Thr	Lys	
<i>E. coli</i>	Val	Gly	Ala	Leu	Ser	Lys	Gly	Gln	Leu	Lys	Glu	Phe	Leu	Asp	Ala	105
<i>Chromatium</i>	Val	Gly	Ala	Val	Ser	Lys	Ser	Gln	Leu	Thr	Ala	Phe	Leu	Asp	Ser	
<i>Chlorobium</i>	Val	Gly	Ala	Leu	Pro	Lys	Asn	Met	Ile	Ala	Lys	Lys	Ile	Asp	Glu	
<i>Rhodospirillum</i>	Ile	Gly	Ala	Leu	Pro	Lys	Thr	Lys	Leu	Phe	Glu	Trp	Val	Glu	Ala	
<i>E. coli</i>	Asn	Leu	Ala													
<i>Chromatium</i>	Asn	Xle														
<i>Chlorobium</i>	His	Ile	Gly													
<i>Rhodospirillum</i>	Ser	Val														

FIG. 2. Amino acid sequence of *R. rubrum* thioredoxin in comparison with counterparts from *E. coli*, *Chromatium vinosum* and *C. thiosulfatophilum*. The sequences of the latter two thioredoxins have been refined with respect to the published data (9, 12) by the differentiation of some of the leucines and isoleucines based on a more recently discovered mass spectrometric fragmentation process (9a). Areas of homology are boxed.

FIG. 1. Amino acid sequence of *R. rubrum* thioredoxin. (a) Trypsin-digested peptides; (b) *S. aureus* protease digestion of selected tryptic peptides; (c) *S. aureus* protease-digested peptides; (d) further digestion of selected *S. aureus* proteolytic peptides with chymotrypsin. —, Sequences derived from fast-atom-bombardment tandem mass spectrometry; —, regions that match observed molecular weights of unsequenced peptides; Xle, position where leucine could not be distinguished from isoleucine. The first ten amino acids were also identified by gas-phase Edman degradation of the intact protein.

R. rubrum thioredoxin also shared several unique areas of homology with its *E. coli* and *Corynebacterium nephridii* (13) counterparts, including a glutamate at position 30, but differed from thioredoxin from other photosynthetic organisms in that position; thioredoxins from *Chromatium vinosum* and *C. thiosulfatophilum* contain aspartate and serine at position 30, respectively, whereas cyanobacteria (6, 8) and chloroplasts (11) contain proline. Such diversity among the photosynthetic bacterial thioredoxins at this position was unexpected in view of the suggestion that the amino acid at this site may be related to thioredoxin function (5). The importance of the amino acid at position 30 to thioredoxin structure and function in the different photosynthetic organisms emerges as an interesting question for the future, especially in view of the overall similarities in the thioredoxin structures. The homology of *R. rubrum* thioredoxin with that of *E. coli* (56 to 58%) is intermediate between the homology of *Chromatium vinosum* thioredoxin with that of *E. coli* (67 to 69%) and the homology of *C. thiosulfatophilum* thioredoxin with that of *E. coli* (44 to 48%). (In calculations of percent homology, the number of *E. coli* thioredoxin residues was used as the divisor. For a given range of percent homology, the lower value was calculated on the assumption that none of the positions where leucine was indistinguishable from isoleucine match, while the higher value was calculated on the assumption that all of them match.) Such a relationship is in agreement with the phylogenetic analysis deduced from rRNA sequence data (14).

It is now clear that the primary structures of thioredoxins from three major groups of photosynthetic bacteria (purple sulfur, green sulfur, and purple nonsulfur) show similarities with one another and with the thioredoxin from *E. coli*. At the same time, the studies have revealed a diversity among the thioredoxins from this group of photosynthetic organisms that has yet to be explained. It is of interest that while the reduction mechanism has been identified as the classical NADP system in the case of the two more-similar thioredoxins (from purple sulfur and purple nonsulfur bacteria), this mechanism remains unknown for the most diverse thioredoxin of this group (from green bacteria).

B.B.B. is the recipient of grant NAGW427 from the National Aeronautics and Space Administration. K.B. is the recipient of Public Health Service grants GM 05472 and RR00317 from the National Institutes of Health and grant NGR 22-009-005 from the National Aeronautics and Space Administration.

LITERATURE CITED

1. Buchanan, B. B. 1980. Role of light in the regulation of chloroplast enzymes. *Annu. Rev. Plant Physiol.* **31**:93-96.
2. Buchanan, B. B., M. C. W. Evans, and D. I. Arnon. 1967. Ferredoxin-dependent carbon assimilation in *Rhodospirillum rubrum*. *Arch. Mikrobiol.* **59**:32-40.
3. Clement-Metral, J. D., J.-O. Höög, and A. Holmgren. 1986. Characterization of the thioredoxin system in a facultative phototroph *Rhodobacter sphaeroides* Y. *Eur. J. Biochem.* **161**:119-126.
4. Cséke, C., and B. B. Buchanan. 1986. Regulation of the formation and utilization of photosynthate in leaves. *Biochim. Biophys. Acta* **853**:43-63.
5. Gleason, F. K. 1986. Thioredoxin in the cyanobacteria: structure and functional homology to thioredoxins from other organisms, p. 21-30. In A. Holmgren, C.-I. Branden, H. Jörnvall, and B.-M. Sjöberg (ed.), *Thioredoxin and glutaredoxin systems: structure and function*. Raven Press, New York.
6. Gleason, F. K., M. M. Whittaker, A. Holmgren, and H. Jörnvall. 1985. The primary structure of thioredoxin from the filamentous cyanobacterium *Anabaena* sp. 7119. *J. Biol. Chem.* **260**:9567-9573.
7. Holmgren, A. 1985. Thioredoxin. *Annu. Rev. Biochem.* **54**:237-271.
8. Ip, S.-M., P. Rowell, A. Aitken, and W. D. P. Stewart. 1984. Purification and characterization of thioredoxin from the N_2 -fixing cyanobacterium *Anabaena cylindrica*. *Eur. J. Biochem.* **141**:497-504.
9. Johnson, R. S., and K. Biemann. 1987. The primary structure of thioredoxin from *Chromatium vinosum* determined by high-performance tandem mass spectrometry. *Biochemistry* **26**:1209-1214.
- 9a. Johnson, R. S., S. A. Martin, K. Biemann, J. T. Stults, and J. T. Watson. 1987. A novel fragmentation process of peptides by collision induced decomposition in a tandem mass spectrometer: differentiation of leucine and isoleucine. *Anal. Chem.* **59**:2621-2625.
10. Johnson, T. C., N. A. Crawford, and B. B. Buchanan. 1984. Thioredoxin system of the photosynthetic anaerobe *Chromatium vinosum*. *J. Bacteriol.* **158**:1061-1069.
11. Maeda, K., A. Tsugita, D. Dalzoppo, F. Vilbois, and P. Schürmann. 1986. Further characterization and amino acid sequence of m-type thioredoxins from spinach chloroplasts. *Eur. J. Biochem.* **154**:197-203.
12. Mathews, W. R., R. S. Johnson, K. L. Cornwell, T. C. Johnson, B. B. Buchanan, and K. Biemann. 1987. Mass spectrometrically derived amino acid sequence of thioredoxin from *Chlorobium*, an evolutionarily prominent photosynthetic bacterium. *J. Biol. Chem.* **262**:7537-7545.
13. Meng, M., and H. P. C. Hogenkamp. 1981. Purification, characterization, and amino acid sequence of thioredoxin from *Corynebacterium nephridii*. *J. Biol. Chem.* **256**:9174-9182.
14. Woese, C. R. 1987. Bacterial evolution. *Microbiol. Rev.* **51**:221-271.