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FINAL TECHNICAL REPORT

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THIOL BIOCHEMISTRY OF PROKARYOTES

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BRIEF SUMMARY OF PROJECT

Low molecular weight thiols play a key role in cellular defenses against oxygen toxicity. Glutathione is the thiol used in higher organisms but we have shown that it is absent in many prokaryotes. Since substantial diversity must have existed in prokaryotes prior to the evolution of cyanobacteria and accumulation of oxygen in the atmosphere, it might be expected that evolutionarily distinct groups of bacteria evolved mechanisms of protection against oxygen toxicity based upon different low molecular weight thiols. The long term goal of this research is to delineate the differences in the thiol biochemistry of prokaryotes and to understand how these differences evolved in response to the shift from anaerobic to aerobic metabolism. Specific aims of this proposed research are: (1) to expand the methods of thiol analysis developed in our laboratory to measure all known biological thiols and to include analyses for thioesters, disufides, and dithiol proteins (thioredoxin); (2) to apply these methods to establish the main low molecular weight thiols produced by all major groups of prokaryotes; (3) to establish the structures of any novel thiols which appear to have wide distribution; (4) to elaborate the occurence of key enzymes, especially disulfide reductases, involved in the metabolism of these thiols.

With respect to the first specific aim, the methodology based upon fluorescent labeling with monobromobimane for analysis of thiols and thiol derivatives has been greatly extended and now includes ion pairig HPLC techniques which permit analysis of low molecular weight thiols that are highly anionic in character, such as coenzyme A, as well as cationic thiols (P1,P7). Methods for specific analysis of the thiol components of thioesters and disulfides proved more difficult to develop than expected owing to the fact that conventional methods used to cleave disulfides also cleave thioesters but selective cleavage based upon hydroxylamine led to a successful method (P4). An analysis for thioredoxin was also developed based upon fluorescent labeling of the two cysteine residues and HPLC analysis after a heat treatment and desalting procedure (P5). To facilitate isolation and identification of thiols of unknown or uncertain structure from biological systems a rapid and efficient method for purification of thiols from biological extracts was developed based upon thiol affinity chromatography and preparative HPLC (P8).

In accomplishing specific aims (2) and (3) we have shown that glutathione is absent in all strictly anaerobic bacteria studied and is produced only by the purple bacteria and the cyanobacteria (P1, P6). Since the purple bacteria are the group most closely related to mitochondria and the cyanobacteria are related to chloroplasts, this suggests that incorporation of glutathione metabolism into eucaryotes could have occurred during the endosymbiosis giving rise to mitochondria and chloroplasts. This hypothesis is supported by the finding that Entamoeba histolytica, a eucaryote lacking both mitochondria and chloroplasts, does not produce glutathione or the enzymes of glutathione metabolism (P2). To further understand mechanisms of protection against oxygen toxicity it was important to ascertain what thiols are used by aerobic bacteria which lack GSH. The Gram positive bacteria and the radiobacteria were found to produce coenzyme A in unusually large amounts (P1) whereas, among the archaebacteria, the halobacteria were shown to produce half of the glutathione molecule, γ-glutamylcysteine, as the major intracellular thiol (P3). A wide variety of unidentified thiols were also detected, but few occurred at high levels and had wide distribution.

The pursuit of objective (4) was confined primarily to studies of the disulfide reductases responsible for maintaining coenzyme A in a reduced state in Gram positive bacteria and for reducing γ-glutamylcysteine disulfide in halobacteria. At least two coenzyme A disulfide reductases have been identified in Bacillus cereus (A1,A5) and a γ-glutamylcysteine disulfide reductase has been detected in halobacteria (P3). The latter has been purified to homogeneity and is presently being characterized (A6, A. R. Sundquist and R. C. Fahey, unpublished); a full report on this enzyme will be submitted in mid-1987.

In conclusion, the present studies have shown that GSH metabolism arose in the purple bacteria and cyanobacteria where it functions to protect against oxygen toxicity. Evidence was obtained indicating that GSH metabolism was incorporated into eucaryotes via the endosymbiosis giving rise to mitochondria and chloroplasts. Aerobic bacteria lacking GSH utilize other thiols for apparently similar functions, the thiol being coenzyme A in Gram positive bacteria and γ-glutamylcysteine in the halobacteria. The thiol biochemistry of prokaryotes is thus seen to be much more highly diversified than that of eucaryotes and much remains to be learned about this subject.

ABSTRACTS AND PAPERS PRESENTED AT MEETINGS

A1. Isolation and Initial Characterization of a Novel Disulfide Reductase from Bacillus cereus. R. C. Fahey and M. Limova, Fed. Proc. 42, 1964 (1983).

A2. Low Molecular Weight Thiols in Halobacteria. G. L. Newton, B. J. Javor, and R. C. Fahey, West Coast Bacterial Physiologists Annual Asilomar Conference, December 15-17, 1984.

A3. Determination of the Thiol Components of Biologically Important Thioesters and Disulfides. S. S. Fenton and R. C. Fahey, Fed. Proc. 44, 1211 (1985).

A4. Fluorescent Labeling of E. coli Thioredoxin by Monobromobimane. P. C. Chinn, V. Pigiet, and R. C. Fahey, Fed. Proc. 44, 1620 (1985).

A5. Partial Purification and Characterization of an NAD(P)H Dependent Coenzyme A Disulfide Reductase from Bacillus cereus. T. D. Chicken and R. C. Fahey, West Coast Bacterial Physiolgists Annual Asilomar Conference, December 13-15, 1985.

A6. Partial Purification and Characterization of a Bis-γ-Glutamylcystine Reductase from Halobacterium halobium. A. R. Sundquist and R. C. Fahey, West Coast Bacterial Physiolgists Annual Asilomar Conference, December 13-15, 1985.

A7. Evolution of Thiol Protective Systems in Prokaryotes. R. C. Fahey and G. L. Newton, Second Symposium on Chemical Evolution and the Origin and Evolution of Life, Moffett Field, California, July 23-26, 1985.

A8. Evolution of Glutathione Metabolism in Phototrophic Microorganisms. R. C. Fahey, R. M. Buschbacher, and G. L. Newton, Fifth ISSOL Meeting and Eighth International Conference on the Origin of Life, Berekely, July 21-25, 1986.

PUBLICATIONS

P1. Occurrence of Low Molecular Weight Thiols in Biological Systems. R. C. Fahey and G. L. Newton, in "Functions of Glutathione - Biochemical, Physiological, and Toxicological Aspects", A. Larsson, et al, eds., Raven Press, New York, pp. 252-260 (1983).

P2. Entamoeba histolytica: A Eukaryote Without Glutathione Metabolism. R. C. Fahey, G. L. Newton, B. Arrick, T. Overdank-Bogart, S. B. Aley, Science, 224, 70-72 (1984).

P3.)-Glutamylcysteine and Thiosulfate Are the Major Low-Molecular-Weight Thiols in Halobacteria. G. L. Newton and B. Javor, J. Bacteriol. 161, 438-441 (1985).

P4. Analysis of Biological Thiols: Determination of Thiol Components of Disulfides and Thioesters. S. S. Fenton and R. C. Fahey, Anal. Biochem. 154, 34-42 (1986).

P5. Determination of Thiol Proteins Using Monobromobimane Labeling and High-Performance Liquid Chromatographic Analysis: Application to Escherichia coli Thioredoxin. P. C. Chinn, V. Pigiet, and R. C. Fahey, Anal. Biochem. 159, 143-149 (1986).

P6. The Evolution of Glutathione Metabolism in Phototrophic Microorganisms. R. C. Fahey, R. M. Buschbacher, and G. L. Newton, J. Mol. Evol., in press (1987).

P7. Determination of Low Molecular Weight Thiols Using Monobromobimane Fluorescent Labeling and High-Performance Liquid Chromatography. R. C. Fahey and G. L. Newton, Methods Enzymol., in press (1987).

P8. Purification of Thiols from Biological Samples. G. L. Newton and R. C. Fahey, Methods Enzymol., in press (1987).