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THE EVOLUTION OF GLUTATHIONE METABOLISM IN PHOTOTROPHIC MICROORGANISMS

Robert C. Fahey, Ralph M. Buschbacher, and Gerald L. Newton

Department of Chemistry, University of California, San Diego,

La Jolla, California 92093

Received

Correspondence to: Robert C. Fahey

Department of Chemistry

University of California, San Diego

La Jolla CA 92093

Phone (619) 452-2163

SUMMARY. Of the many roles ascribed to glutathione (GSH) the one most clearly established is its role in the protection of higher eucaryotes against oxygen toxicity through destruction of thiol reactive oxygen biproducts. If this is the primary function of GSH then the evolution of GSH metabolism should have occurred during or after the evolution of oxygenic photosynthesis. The fact that many bacteria do not produce GSH is consistent with this view. In the present study we have examined the low molecular weight thiol composition of a variety of phototrophic microorganisms in order to ascertain how evolution of GSH production is related to the evolution of oxygenic photosynthesis. Cells were extracted in the presence of monobromobimane (mBBr) to convert thiols (RSH) to fluorescent derivatives (RSmB) which were analyzed by HPLC. Significant levels of GSH were not found in the green bacteria (Chlorobium thiosulfatophilum and Chloroflexus aurantiacus). Substantial levels of GSH were present in the purple bacteria (Chromatium vinosum, Rhodospirillum rubrum, Rhodobacter sphaeroides, and Rhodocyclus gelatinosa), the cyanobacteria (Anacystis nidulans, Microcoleus chthonoplastes S.G., Nostoc muscorum, Oscillatoria amphigranulata, Oscillatoria limnetica, Oscillatoria sp. (Stinky Spring, Utah), Oscillatoria terebriformis, Plectonema boryanum, and Synechococcus lividus), and in eucaryotic algae (Chlorella pyrenoidsa, Chlorella vulgaris, Euglena gracillis, Scenedesmus obliquus, and Chlamydomonas rheinhardtii). Other thiols measured included cysteine, γ-glutamylcysteine, thiosulfate, coenzyme A, and sulfide; several unidentified thiols were also detected. Many of the organisms examined also exhibited a marked ability to reduce mBBr to syn-(methyl,methyl)bimane, an ability which was quenched by treatment with 2-pyridyl disulfide or 5,5'-bisdithio-(2-nitrobenzoic acid) prior to reaction with mBBr. These observations indicate the presence of a reducing system capable of electron transfer to mBBr and reduction of reactive disulfides. The distribution of GSH in phototrophic eubacteria indicates that GSH synthesis evolved at or around the time that oxygenic photosynthesis evolved.

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evolved.

KEY WORDS. Glutathione - Evolution - Phototrophic Microorganisms - Oxygen Toxicity

INTRODUCTION

Glutathione has been postulated to play a key role in many biological processes (Jocelyn, 1972; Kosower and Kosower, 1978; Meister and Anderson, 1983) but its function in protecting cells against the toxicity of oxygen and other thiol reactive agents is the role which has been most thoroughly established. Key enzymes involved in this protection against oxygen toxicity in animals are glutathione peroxidase (Wendel, 1980), which catalyzes the destruction of hydrogen peroxide and organic hydroperoxides (Eq 1), and the glutathione S-transferases (Chasseaud, 1976; Jakoby and Habig, 1980), which catalyze the destruction of hydroperoxides and the conjugation of GSH with epoxides, enones, and other sulfhydryl reactive agents.

$$2GSH + H_2O_2 = GSSG + 2H_2O \quad (1)$$

Glutathione peroxidase activity has not been found in plants and it is thought (Halliwell, 1981) that ascorbate oxidase fulfills an analogous function with the dehydroascorbate formed in the destruction of H_2O_2 (Eq 2) being reduced by GSH back to ascorbate (Eq 3).

$$\text{ascorbate} + H_2O_2 = \text{dehydroascorbate} + 2H_2O \quad (2)$$

$$2GSH + \text{dehydroascorbate} = GSSG + \text{ascorbate} \quad (3)$$

Glutathione reductase catalyzes the reduction of the GSSG formed in reactions 1 and 3 back to GSH using NADPH as the reductant (Eq 4).

$$GSSG + NADPH + H^+ = 2GSH + NADP^+ \quad (2)$$

Under normal conditions the latter reaction maintains the GSH:GSSG ratio at a value generally in excess of ~50 and insures an environment in which the essential sulfhydryl groups of key enzymes and coenzymes are protected (Kosower and Kosower, 1978).

If the primary function of glutathione is oxygen detoxification, then glutathione metabolism presumably arose at the time oxygen was accumulating as the consequence of the evolution of oxygenic photosynthesis. Considerable diversification must have existed among the bacteria extant on the earth at the time that oxy-

gen became a significant component of the atmosphere and it would be surprising if the only thiol-based protection system to evolve was that based upon GSH. The finding that many bacteria, including some strict aerobes, lack GSH but contain other low molecular weight thiols is consistent with the idea that more than one thiol protection system has evolved in procaryotes (Fahey et al, 1978). Thiol analysis of bacteria lacking GSH has suggested that Coenzyme A may be the key compound in the thiol protecting systems of some aerobic Gram positive bacteria (Fahey and Newton, 1983) whereas γ-glutamylcysteine appears to form the basis for such a system in halobacteria (Newton and Javor, 1985). Glutathione appeared to occur primarily in the aerobic and facultative Gram negative bacteria but not in anaerobes or in Chromation vinosum based upon results of enzymatic assay for glutathione (Fahey et al, 1978).

In the present study we utilize recently developed methods to analyze the low molecular weight thiols in a variety of phototrophic microorganisms selected to represent different stages in the evolution of the metabolism necessary for oxygenic photosynthesis and different levels of tolerance and utilization of oxygen. We survey selected representatives of the green bacteria, of all main branches of the evolutionary tree for purple photosynthetic bacteria as derived from 16s rRNA sequence data (Fox et al, 1980), and of the cyanobacteria, the latter constituting the only group of prokaryotes capable of oxygenic photosynthesis. The objective was to pinpoint more carefully which groups produce GSH in order to establish a basis for understanding the origin of glutathione metabolism. A selection of eucaryotic algae was also examined to further test the generality of occurrence of GSH among eucaryotes.

EXPERIMENTAL

Organisms. Photoautotrophic growth conditions were used for growth wherever possible. When heterotrophic growth was used the medium was tested for the presence of glutathione and media containing yeast extract were found to contain high levels of glutathione. When the level found was $\geq$ 0.5 uM, the glutathione was specifically depleted as described previously (Fahey et al, 1984). Cells were harvested in late log phase by centrifugation and aliquots were used immediately or stored at -80° until extracted.

The samples of the following organisms were kindly provided by D.E. Carlson, T. Johnson, and B.B. Buchanan. Chlorobium thiosulfatophilum (Tassajara) was grown anaerobically and photoautotrophically in the medium described by Buchanan, et al (1972). Chromatium vinosum D was cultured anaerobically and photoautotrophically in a CO_2-thiosulfate medium described by Arnon et al (1963). Nostoc muscorum 7119 was cultured photoautotrophically as described by Arnon et al (1974). Chlorella vulgaris was cultured photoautotrophically according to Arnon et al (1955) with the E7 and Fe-EDTA solutions of Arnon et al (1963), and was grown heterotrophically in the dark on this medium supplemented with 0.2% glucose. Euglena gracilis Z was cultured photoautotrophically according to Edmunds (1965) and 0.2% glucose was added to this medium for dark, heterotrophic growth.

Samples of the following organisms were generously provided by R. Fall. Plectonema boryanium UTEX 594 was photoautotrophically grown as described by Stratton et al (1979). Chlorella pyernoidosa UTEX 1230 and Scenedesmus obliquus UTEX 1450 were cultured photoautotrophically as described by Sorokin and Krauss (1958).

Chloroflexus aurantiacus ATCC 29362 was cultured anaerobically and photoheterotrophically on ATCC medium 920 (glutathione depleted). Rhodospirillum rubrum ATCC 11170, via B. Bartsch, was cultured anaerobically and photohetero-

trophically on malate (Ormerod, et al, 1961). Dark heterotrophic growth on malate in the presence of air was accomplished using the medium of Ormerod et al (1961). Anaerobic, photoheterotrophic growth of Rhodobacter sphaeroides (formerly, Rhodopseudomonas sphaeroides) ATCC 550 was carried out on medium S described by Lascelles (1956), and of Rhodocyclus gelatinosa (formerly, Rhodopseudomonas gelatinosa) ATCC 17011 on ATCC medium 112, the media being depleted of glutathione in both cases. Chlamydomonas reinhardtii 137Cm+ was kindly supplied by S. Howell and was grown photoautotrophically on the high salt medium (HSM) of Sueoka et al (1967).

Barbara Javor kindly provided samples of Oscillatoria terebriformis OH-80-Ot-D grown to stationary phase at 45° in D medium (Castenholz, 1981) buffered with 5 mM Hepps, of Synechococcus lividus OH-53-S harvested in log phase after grown on DG medium (D medium plus 0.8 g/L glycylglycine buffer) at 45°, of Anacystis nidulans 625 cultured in DG medium at 27°, and of Oscillatoria amphigranulata WT-RC harvested in log phase after growth at 45° in D medium lacking KNO_3 and $NaNO_3$ but with addition (g/L) of Na_2HPO_4 (0.07), KH_2PO_4 (0.036), tricine (0.9) and NH_4Cl (0.2).

Yehuda Cohen generously furnished samples of Oscillatoria limnetica (Solar Lake, Sinai) cultured 7 days at 40° in CHU 11 medium (Cohen, et al, 1975). He also provided Oscillatoria sp. isolated from Stinky Spring, Utah and grown 7 days at 40° in ASNN III medium (Rippka, et al, 1979). Also provided was Microcoleus chthonoplastes S.G. from Spencer Gulf, South Australia which was cultured 7 days at 40° in the ASN III medium above but with the NaCl, $MgSO_4 \cdot 7H_2O$, $MgCl_2$, and KCl at double the given concentrations. Further details are found in Cohen, et al (1986).

Materials. Glacial acetic acid and (ethylenedinitrilo)tetraacetic acid disodium salt (EDTA) were obtained from Mallincrodt, HPLC grade acetonitrile and

methanol were from Burdick and Jackson, and 2,2'-dithiodipyridine and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were from Sigma. Monobromobimane (mBBr) and N-2-hydroxyethylpiperazine-n'-3-propanesulfonic acid (HEPPS) were from Calbiochem. Yeast extract was from Difco and all other culture media components were of reagent grade or higher purity.

Sample Preparation. The cell extracts were prepared using a minor modification of the method of Fahey and Newton (1983). A frozen cell pellet (~200 mg wet weight) was flushed for 10 min with argon in a 3 ml septum capped vial after which 0.5 ml of argon saturated aqueous 100 mM HEPPS (pH 8.0) containing 5 mM EDTA and 2 mM mBBr was added and the mixture allowed to stand 5 min. Argon saturated acetonitrile (0.5 ml) was then added and the sample incubated at 60°C for 15 min. The vial was opened, the sample homogenized 1 min with a Tekmar Tissuemizer, glacial acetic acid added to 1%, and the sample cooled on ice. Protein and cell debris were removed by centrifugation for 5 min in an Eppendorf Microfuge. The sample was diluted 1:1 with aqueous 1% acetic acid and recentrifuged prior to HPLC analysis. The acetonitrile pellets were dried and weighed to obtain the residual dry weight (~80% of the total dry weight). For control samples 2,2'-dithiodipyridine (2 mM) or DTNB (2 mM) was substituted for mBBr during homogenization. Just before addition of acetonitrile, mBBr was added to 4 mM and allowed to react 5 min at room temperature. Further processing of control samples was as described above.

HPLC Chromatography. A combination of two HPLC protocols was used to achieve full resolution of the low molecular weight thiols. The reverse phase protocol (Method 1) described by Fahey and Newton (1986) resolved most of the compounds listed in Tables 1-3. However, cysteine and thiosulfate, which elute near 6 min, are poorly separated, and Coenzyme A elutes at 45 min as a peak too broad to detect at low CoA levels. Coenzyme A elutes as a sharp peak at 26 min and is

easily quantitated using a tetrabutylammonium ion pairing system (Method 2, Fahey and Newton, 1986), and cysteine and thiosulfate were also well resolved in this protocol, eluting at 6 and 21 min, respectively. The use of these two different protocols for analysis of all samples provided reasonable assurance as to the correctness of the peak assignments.

RESULTS

Extraction of cells was carried out by suspending them in pH 8 buffer containing mBBr and subsequently deproteinizing by adding an equal volume of acetonitrile and heating. Since mBBr penetrates cell membranes this approach was thought to provide maximal derivatization of thiols prior to their exposure to oxygen. This conclusion appeared justified by the finding that extraction of most cells with degassed buffer under argon gave essentially the same result as extraction in air. When recoveries were tested by adding known mixture containing thiosulfate, sulfide, cysteine, γ-Glu-Cys, GSH, and CoA, all at 1 umole per g, recoveries were generally acceptable (~80%). However, for Chromatium vinosum recoveries as low as ~50% were found for some thiols when extraction was conducted in air but higher recoveries (~80%) where obtained when extraction took place under argon. Thus, the protocol involving extraction under argon is recommended. Since this procedure was not used for all samples the present results should be considered to be semiquantitative. A second sample was extracted with 2-pyridyl disulfide used in place of mBBr in the extraction buffer, with mBBr being added after the cells were lysed. Since 2-pyridyl disulfide oxidizes thiols and prevents their labeling by mBBr, this sample served as a control used to identify nonthiol fluorescent components present in the cell or derived from mBBr.

The results obtained are summarized in Tables 1-3. The process used to arrive at these results is illustrated for the case of Chromatium vinosum. HPLC chromatograms obtained using Method 1 are presented in Fig. 1 and by Method 2 in Fig. 2. Comparison of the chromatogram for the derivatized extract (Fig 1B) with that of the corresponding control sample (Fig. 1A) reveals a number of peaks that dissapear upon treatment with 2-pyridyl disulfide. The peaks corresponding to thiosulfate, cysteine, and γ-glutamylcysteine were quite small at the amplification shown and are not labeled; the position at which they elute is seen in the

chromatogram of known thiol derivatives shown in Fig. 1C. At higher gain they were more evident and the presence of cysteine was verified in the analysis using Method 2 (Fig. 2A). The peaks for thiosulfate and γ-glutamylcysteine are masked by other substances in Fig. 2A.

The next major thiol derivative apparent in Fig. 1B was that of GSH and its presence was confirmed as shown in Fig 2A. Following the GSH derivative (Fig. 1B) was a peak that could not be assigned to any of known thiols previously characterized (Fahey and Newton, 1983) and was designated U14. It eluted most closely to the derivative of homocysteine using Method 1 but addition of an authentic sample of the mBBr derivative of homocysteine resulted in a double peak when the sample was chromatographed using Method 2 (not shown) so that homocysteine was excluded. U14 eluted at approximately 8 min under Method 2 (Fig. 2A).

A major peak was observed to elute at 34 min under protocol A (Fig. 1B) and at 16 min under protocol B (Fig. 2A). This did not correspond to any of the thiols previously characterized and extraction with ethyl acetate removed this derivative from the mixture. The elution times correspond to those of syn-(methyl,methyl)bimane, the compound formally resulting from displacement of the bromide in mBBr by hydride, and addition of authentic syn-(methyl,methyl)bimane to the sample gave HPLC chromatograms exhibiting no double peaking. The green and purple sulfur bacteria all produced syn-(methyl,methyl)bimane in large amounts. The purple nonsulfur bacteria, the cyanobacteria (Table 2), and the phototrophic eucaryotes (Table 3) studied also produced syn-(methyl,methyl)bimane but in amounts that varied widely with the specific species. Lesser amounts were observed for Plectonema boryanum, Euglena gracilis, and light grown Chlorella vulgaris than for the other organisms listed. Since syn-(methyl,methyl)bimane was not produced in the control sample it is evident that the source of the electrons leading to its formation must be destroyed by 2-pyridyl disulfide.

Coenzyme A elutes as a broad peak around 46 min under Method 1 and was not detected in Fig. 1B. The presence of CoA in Chromatium vinosum was clearly indicated by the HPLC analysis using Method 2 (Fig. 2A) and the quantitative data for CoA presented in Tables 1-3 were obtained using this procedure. A small peak for dephospho-CoA was also seen (Fig. 2A) but its formation from CoA during the extraction process has not been excluded and quantitative data for dephospho-CoA were not included in Table 1.

Two peaks appeared at 49 and 54 min which were partly, but not completely, removed in the control sample (Figs. 1A,B). The structure and origin of these components is not understood. They were not found in extracts of the other organisms listed in Table 1. The derivative of H_2S elutes between these two peaks at 52 min using Method 1 (Fig. 1) and at 22 min under Method 2 (Fig. 2). Sulfide was the least reproduceable of the thiol components measured. In the control samples a large peak appears at 59 min which corresponds to the mBBr derivative of 2-mercaptopyridine. A second smaller peak, which eluted at 54 min, was seen in all control samples and is thought to derive from an impurity in the 2-pyridyl disulfide. The use of DTNB was found preferable to 2-pyridyl disulfide in that it gives rise to only one mBBr derivative with low fluorescence which eluted at 44 min (Method 1) and DTNB was therefore used to generate control samples for many of the organisms studied.

One disturbing feature of the results was the high cysteine levels seen in some organisms. We were concerned that the GSH derivative might be degraded by peptidases under the extraction conditions leading to false values for cysteine. To test this we extracted Euglena gracilis in methanesulfonic acid to fully denature enzymes (Fahey and Newton, 1983). Neutralization in the presence of mBBr, deproteinization, and HPLC analysis yielded values for cysteine essentially identical to those of Table 1 so that formation of cysteine via enzymatic degradative

processes can be excluded.

DISCUSSION

The results of the present study show that the ability to synthesize GSH occurs among a wide range of phototrophic eubacteria. The green bacteria represent the only group in which the occurrence of glutathione is questionable. The low level seen in Chlorobium thiosulfatophilum cannot be ignored but the levels of cysteine, thiosulfate, CoA, sulfide, and the unknown U11 all clearly exceed the level of GSH in Chlorobium. No significant amount of GSH could be deteted in Chloroflexus aurantiacus. Since most green bacteria are obligate anaerobes (Chloroflexus being an exception which can grow aerobically) the absence of significant levels of GSH in these organisms is expected if the fundamental function of GSH is to protect against oxygen toxicity. If GSH does actually occur at very low levels in the green bacteria its function there must clearly be different from that in organisms producing GSH at high levels.

Glutathione is clearly present in the purple sulfur bacterium Chromatium vinosum which tolerates oxygen but does not grow in air (Gibson, 1967). In our previous survey of glutathione in bacteria we were unable to detect glutathione in Chromatium vinosum (Fahey et al, 1978). The reason for this discrepancy is not clear. The sample used in the earlier study had been stored frozen for an extended period so that GSH might have oxidized but the enzymatic assay used to measure glutathione involves a cycling method in which GSSG is reduced by glutathione reductase and reoxidized by tetrathionate (Fahey et al, 1975) so that GSSG should have been detected. Oxidation to a form not reduceable by glutathione reductase or other degradative processes may have reduced the apparent glutathione level below that detectable by the enzymatic assay. A glutathione reductase has been isolated from Chromatium vinosum but has a rather high K_m (7 mM) for GSSG (Chung and Hurlbert, 1975) and it seems possible that GSSG is not the preferred

substrate of this enzyme. Since U14 and CoA occur at comparable levels to GSH in Chromatium (Table 1) it would be of interest to test the disulfides of these as alternative or additional sources of substrates for this enzyme. It is noteworthy that the glutathione reductase from Chromatium utilizes NADH in preference to NADPH which characterizes it as substantially different from other glutathione reductases (Chung and Hurlbert, 1975). Amino acid sequence data show that glutathione reductase and lipoamide dehydrogenase are related (Williams, 1976) and, based on the wider distribution of lipoamide dehydrogenase than of GSH among procaryotes, it has been suggested that glutathione reductase evolved from lipoamide dehydrogenase (Fahey, 1977). Since lipoamide dehydrogenase utilizes NADH, the finding of an NADH-linked glutathione reductase in Chromatium suggests that Chromatium may typify an early stage in the evolution of glutathione metabolism, as view which is in accord with the fact that chromatium is the least oxygen-adapted organism tested here to produce GSH at substantial levels. Further studies of GSH metabolism in this organism are therefore likely to be informative.

Quite high levels of GSH were also found in the purple nonsulfur bacteria (Table 1) which is of special significance since this group has oxygen-dependent metabolism and is closely related to the mitochondria of eucaryotes. More specifically, nucleotide sequencing of 5S ribonosmal RNAs shows that members of the alpha subgroup of the purple photosynthetic bacteria, which includes Rhodospirillum rubrum and Rhodobacter sphaeroides, are the ones most closely related to wheat mitochondria (Villanueva, et al, 1985). Thus, the endosymbiotic process (see Margulis, 1981) giving rise to mitochondria would presumably have incorporated the ability to produce GSH into eucaryotes. The beta (Rhodocyclus gelatinosa) and gamma (Chromatium vinosum and E. coli) subgroups of the purple photosyntheic bacteria also have the capacity to synthesize GSH. Glutathione reductase appears to be widely distributed among these groups, having been purified from Chromatium

vinosum (Chung and Hurlbert, 1975), Rhodospirillum rubrum (Boll, 1969), and E. coli (Williams and Arscott, 1971), and assayed in other purple bacteria and relatives (Ondarza, et al, 1983). The purple bacteria were found to have generally high levels of CoA suggesting that CoA may serve along with GSH in a protective fashion in the purple bacteria. This possibility is strengthened by the finding that Rhodospirillum rubrum extracts contain comparable GSSG and CoASSG reductase activities (Ondarza, et al, 1983).

All of the cyanobacteria studied, including representatives of all four classes recognized by Cohen, et al (1986) based upon sulfide sensitivity and ability to carry out anoxygenic photosynthesis, produce high levels of GSH (Table 2). In contrast to the purple bacteria, CoA levels were quite low in all of the cyanobacteria and in this regard it is interesting that the cyanobacterium Spirilum maxima was found to exhibit very high GSSG reductase activity but no detectible CoASSG reductase activity (Ondarza, et al, 1983). The cyanobacteria are closely related to the eucaryotic chloroplast based upon RNA sequence data (Fox, et al, 1980) so that the endosymbiotic process giving rise to chloroplasts represents a second mechanism by which the ability to synthesize GSH could have been incorporated into phototrophic eucaryotes. Since the purple bacteria and the cyanobacteria represent the only two groups of bacteria which we have uniformly found to produce GSH, the endosymbioses leading to mitochondria and chloroplasts represent the most plausible mechanisms for incorporation of GSH synthesis into eucaryotes. Entamoeba histolytica represents the only eucaryote clearly demonstrated not to produce GSH. This, coupled with the fact that this organism lacks both chloroplasts and mitochondria, supports the view that GSH metabolism evolved in eucaryotes via the endosymbioses giving rise to mitochondria and chloroplasts (Fahey, et al, 1984).

All of the eucaryotic phototrophs examined were found to produce GSH (Table

3). However, the level was markedly higher in photoautotrophically grown cells than in dark, heterotrophically grown cells, an effect not seen in Rhodospirillum rubrum (Table 1). The reason for this variation is not understood.

The results of the present study appear to support the view that GSH synthesis evolved in phototrophic eubacteria before or around the time that oxygenic photosynthesis evolved. It is therefore tempting to postulate that the initial function of GSH was to protect against oxygen toxicity but there is little other evidence to support this idea. Although glutathione reductase is widely found in these bacteria (see above) relatively little is known about other enzymes of GSH metabolism. GSH appears to play a protective role in E. coli, a relative of the purple bacteria, based upon studies of mutants blocked in GSH synthesis (Apontoweil and Berends, 1975; Fuchs and Warner, 1975; Murata and Kimura, 1982), but it has been reported that GSH peroxidase is absent (Smith and Shrift, 1979) and that GSH S-transferase is present at only a low level (Lau, et al, 1980) in E. coli. Since these are key enzymes of oxygen detoxification by GSH in higher organisms, their apparent absence in E. coli raises serious question about the role of GSH in this organism. Glutathione S-transferase activity was also not found in R. rubrum (Morgenstern, et al, 1986) but little else is known concerning the detoxifying enzymes of GSH metabolism in phototrophic eubacteria. It is possible that GSH played some entirely different function in early eubacteria and that the oxygen detoxification function evolved only later. More detailed information on the distribution of the enzymes which utilize GSH in the purple sulfur and cyanobacteria is needed to clarify the role of GSH in these organisms.

The only phototrophic bacteria which tolerate oxygen and are known not to produce GSH are the halobacteria (Newton and Javor, 1985). However, these bacteria, members of the archaebacteria (Fox, et al, 1980), produce γ-glutamylcysteine, half the GSH molecule, in large amounts and have a disulfide

reductase which maintains it in the reduced state (Newton and Javor, 1985). The halobacteria do not use photosystems I or II but employ a unique bacteriorhodopsin-containing membrane to derive ATP from light. The evolution of a system based upon γ-glutamylcysteine apparently similar to that based upon GSH in these organisms suggests that there are unique features of the γ-glutamylcysteine moiety which are important in aerobic phototrophic organisms. Further studies of the role of γ-glutamylcysteine in the halobacteria are needed to clarify it function in these organisms.

The formation of syn-(methyl,methyl)bimane from mBBr by illuminated spinach chloroplasts and by direct electrochemical reduction has been described by Melis, et al (1986). They ascribe its formation with chloroplasts to a reduction involving photosystem II. The present finding that syn-(methyl,methyl)bimane is formed from mBBr in extracts of the purple bacteria, the cyanobacteria and the eucaryotic algae is consistent with this view. However, we have observed the formation of syn-(methyl,methyl)bimane from mBBr in extracts of various nonphotosynthetic bacteria including those of Streptococcus mutans and Spirochaeta halophila (G. Newton and R. Fahey, unpublished) so that the process is not restricted to phototrophic organisms. The factor or factors responsible for such reduction require further study.

Finally, we note that the unidentified thiol, Ull, appears to be widely distributed among phototrophic bacteria and algae so that its structure and function clearly merits further investigation.

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CAPTIONS TO FIGURES

Figure 1. HPLC chromatograms obtained using Method 1: (A) Control sample for Chromatium vinosum. (B) Chromatium vinosum extracted in the presence of mBBr; (C) Standard mixture. Abbreviations: C - peaks present in control samples; Pys - peaks derived from 2-pyridyl disulfide reagent; U14 - unidentified thiol derivative eluting at 14 min under protocol A; syn-(CH_3,CH_3)B - syn-(methyl,methyl)bimane; γ-GLUCYS - γ-glutamylcysteine; R - peaks derived from buffer and reagents; other abbreviations defined in text.

Figure 2. HPLC chromatograms obtained using Method 2: (A) Chromatium vinosum extracted in the presence of mBBr; (B) Standard mixture. Abbreviations: dpCoA - dephosphocoenzyme A; others as in caption to Fig. 1.

Table 1. Thiols in phototrophic eubacteria

Organism	Thiols Found (umoles per g res. dry weight) Cys	γ-Glu-Cys	GSH	$SSO_3^=$	CoA	H_2S	Unknowns[a]
Green Sulfur Bacteria							
Chlorobium thiosulfatophilum	0.088	≤0.002	0.040	0.84[b]	0.18	1.1	0.08 (U11)
Chloroflexus aurantiacus	≤0.003	≤0.003	≤0.014	1.9	0.19	0.4[b]	1.1 (U11), 0.6 (U16)
Purple Sulfur Bacteria							
Chromatium vinosum	0.046	0.11	1.4	0.09	0.7	2.2	1.5 (U14)
Purple Nonsulfur Bacteria							
Rhodospirillum rubrum							
light grown	0.32	0.28	5.0	≤0.05	1.2	2.0	
dark grown	0.58	0.17	5.0	≤0.05	0.58	0.5	
Rhodobacter sphaeroides	0.40	0.22	24	≤0.12	1.6	1.4	
Rhodocyclus gelatinosa	0.45	0.41	15	0.26	0.61	2.1	

[a]U11 designates an unidentified thiol derivative having a retention time of 11 min; yields of unidentified thiols based upon the fluorescence yield factor for GSmB.

[b]Present in the growth medium.

Table 2. Thiols in cyanobacteria

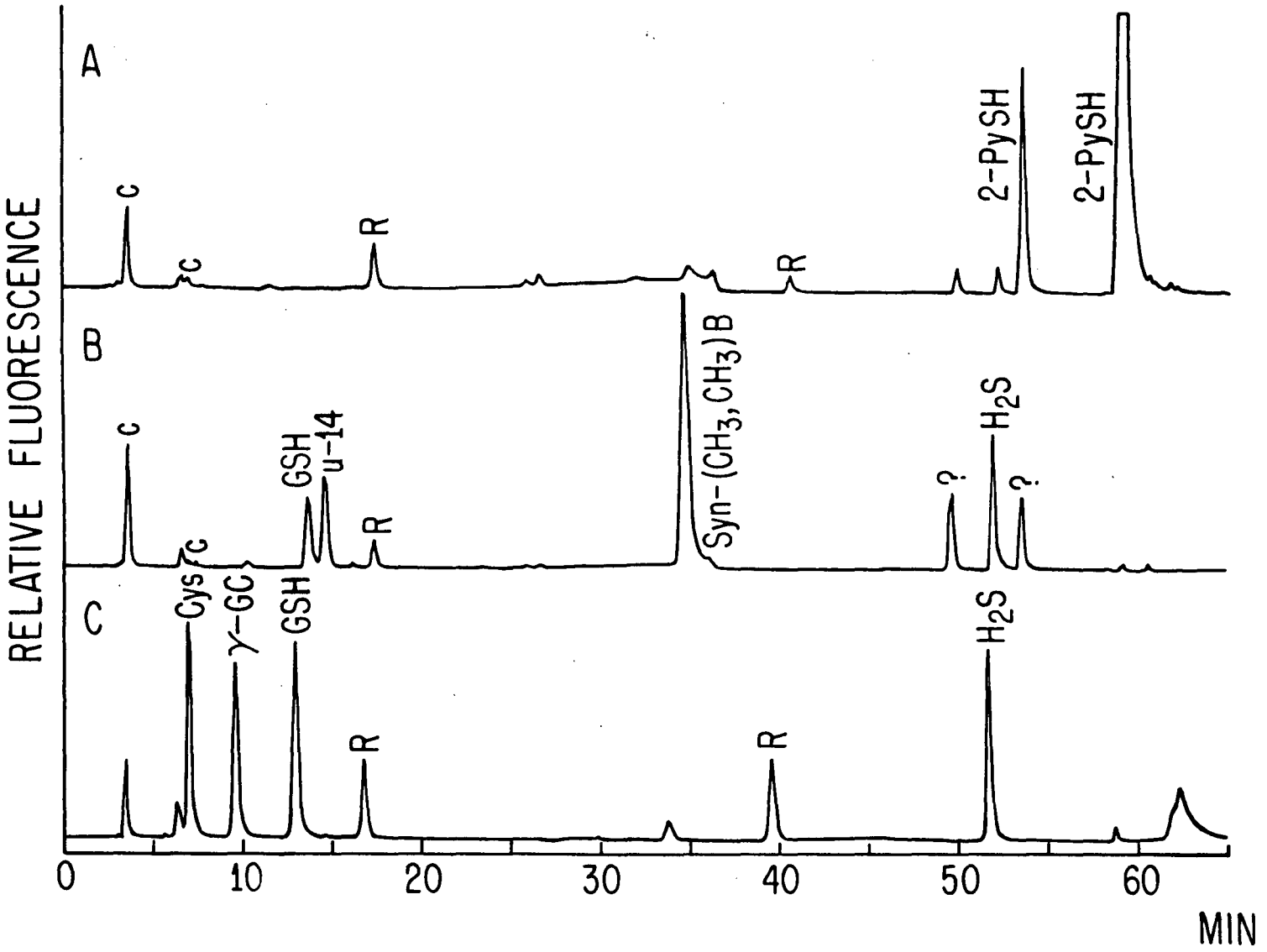
Organism	Thiols Found (umoles per g res. dry weight) Cys	γ-Glu-Cys	GSH	$SSO_3^=$	CoA	H_2S	Unknowns[a]
Anacystis nidulans	≤0.06	≤0.04	>6	0.063	≤0.004	3.4	
Nostoc muscorum	2.0	1.6	0.79	≤0.01	≤0.03	1.7	
Plectonema boryanum	≤0.01	≤0.01	0.7	≤0.06	≤0.03	1.7	
Synechococcus lividus	0.64	≤0.014	1.5	0.09	≤0.02	4.6	
Microcoleus chthonoplastes	≤0.01	0.033	1.0	≤0.03	≤0.001	0.5	
Oscillatoria amphigranulata	0.17	≤0.01	0.40	0.16	≤0.01	2.0	
Oscillatoria limnetica	≤0.01	0.071	4.9	≤0.05	0.024	0.06	
Oscillatoria sp. (Stinky Spring, Utah)	≤0.02	0.031	3.5	≤0.05	0.03	0.4	
Oscillatoria terebriformis	0.17	≤0.11	6.5	≤0.03	≤0.09	1.5	1.8 (U11)

[a]U11 designates an unidentified thiol derivatives having a retention time of 11 min.

Table 3. Thiols in algae and chloroplasts

Organism	Thiols Found (umoles per g res. dry weight)						
	Cys	γ-Glu-Cys	GSH	$SSO_3^=$	CoA	H_2S	Unknowns[a]
Chlorella pyrenoidsa							
light grown	0.15	0.09	7.2	≤0.05	0.06	0.14	
dark grown	0.42	≤0.05	1.3	≤0.05	0.22	0.7	
Chlorella vulgaris							
light grown	0.38	0.15	10	≤0.02	0.16	1.4	
dark grown	0.98	0.10	1.9	0.11	0.35	1.5	
Euglena gracillis							
light grown	6.3	0.3	1.8	≤0.05	0.41	0.9	0.04 (U11)
dark grown	12	≤0.001	0.8	≤0.05	0.02	0.4	
Scenedesmus obliquus	≤0.003	≤0.003	0.48	≤0.003	≤0.002	≤0.01	
Chlamydomonas rheinhardtii	0.28	≤0.01	0.35	≤0.01	0.057	0.6	0.04 (U11)

[a]U11 designates an unidentified thiol derivatives having a retention time of 11 min.


A
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C
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c
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Syn-(CH3,CH3)B
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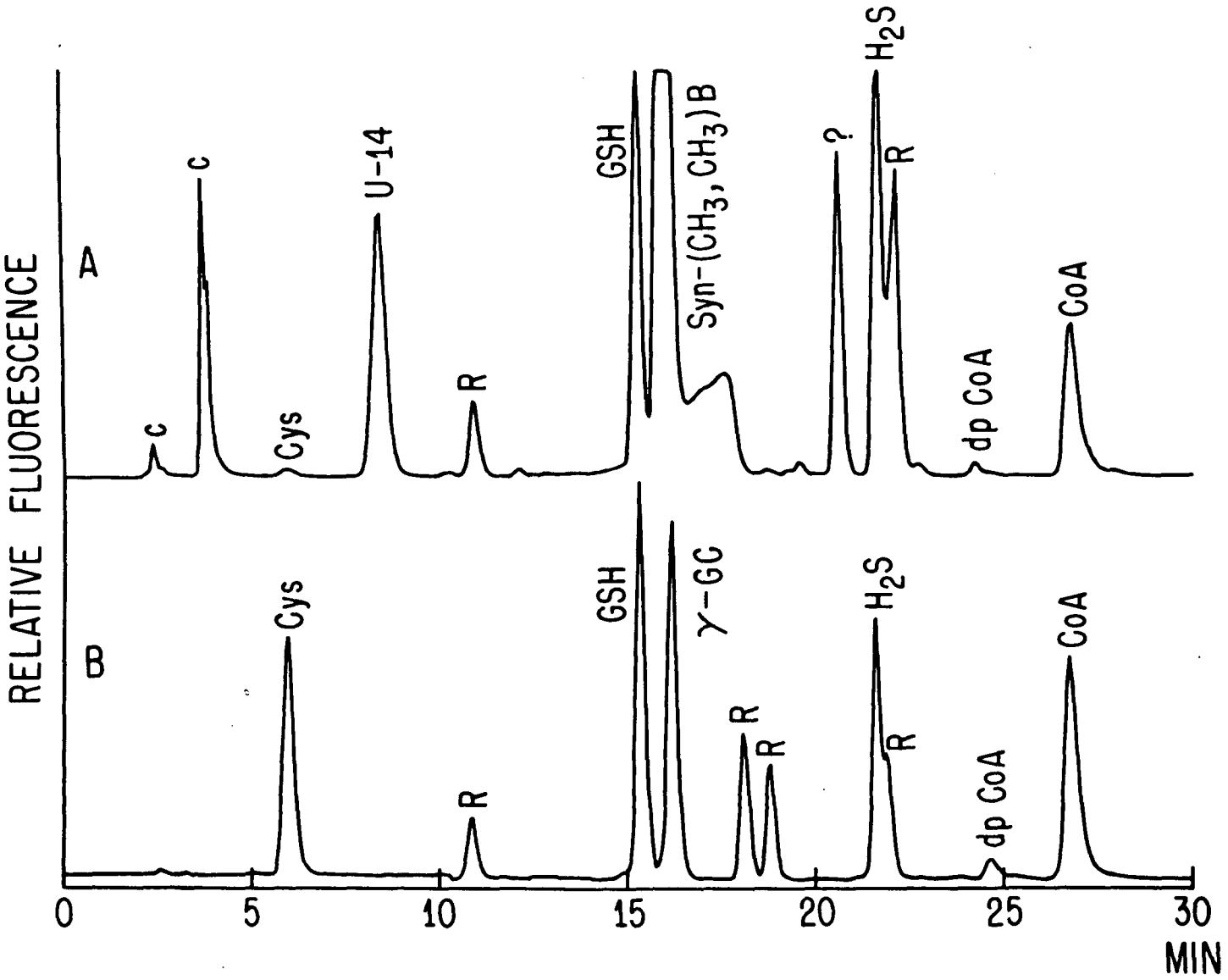
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