Vol. 112, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1983 Pages 66-72

ORIGIN OF THE LABILE SULFIDE IN THE IRON-SULFUR PROTEINS OF ESCHERICHIA COLI

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Received February 23, 1983

SUMMARY. A method has been devised for measuring the abundance of sulfur-34 in the hydrogen sulfide released upon the acidification of Escherichia coli cells. Evidence is presented, based on the rate at which the hydrogen sulfide is released from the cells as well as the total amount released, that this hydrogen sulfide originates from the iron-sulfur proteins present in the cells. The sulfur-34 abundance in this hydrogen sulfide which was isolated from cells grown with [sulfane-^{34}S]thiocystine, a compound which can differentially label in vivo the sulfur-34 abundance of cysteine and hydrogen sulfide, shows cysteine sulfur and not hydrogen sulfide to be the origin of the sulfide sulfur of iron-sulfur proteins in aerobically grown E. coli.

Much information has been gathered on the physical and chemical properties of iron-sulfur proteins since their discovery over two decades ago. However, we still know little about the biological formation of the iron-sulfur centers in these important and perhaps very primitive electron transport proteins (1).

An important initial step in solving this problem is to determine the source of the sulfide or "labile" sulfur used in the production of the iron-sulfur centers of these proteins. Considering what is currently known about the bacterial metabolism of reduced inorganic sulfur there would appear to be two logical choices. Either it originates directly from free inorganic sulfide present in the cell or it is derived from an organic source, i.e., cysteine.

Work, primarily with ferredoxins, has shown that it is possible to chemically form the iron-sulfur center of these proteins by incubation of the apoprotein with ferrous ions and sulfide (2,3,4,5). This work gives

0006-291X/83 $1.50

support to the idea that inorganic sulfide is the sulfur source used in the production of the sulfide in these proteins in vivo. This idea is further supported by the work of Jeng and Mortenson who demonstrated the enzyme catalyzed exchange of the iron-sulfur sulfide of ferredoxin with inorganic sulfide (6). Taniguchi and Kimura, on the other hand, suggested that the sulfide of the iron-sulfur proteins of the adrenal cortex originates from cysteine by the sequential action of cysteine transamination and 3-mercapto-pyruvate sulfur transferase (7). Other workers have postulated that rhodonase may be an important compound in the enzyme system which supplies the sulfide for iron-sulfur proteins (8,9).

In order to resolve the question of whether the sulfide sulfur of iron-sulfur proteins comes from inorganic sulfide or from an organic source such as cysteine, E. coli cells were grown with [sulfane-^{34}S]thiocystine and the extent of labeling of the iron-sulfur protein sulfide was compared with that found in the cysteine and the inorganic sulfide present in the cells. Previous work (10) has shown that in cells grown with this substrate as the sole sulfur source, it is possible to label to different extents with sulfur-34 the cysteine and hydrogen sulfide present in the cells thus allowing for the distinction between these two sulfur sources.

The methods developed in order to conduct this experiment are described herein along with the experimental results which show that an organic sulfur compound, presumably cysteine and not hydrogen sulfide, supplies the bulk of sulfide for the iron-sulfur proteins in aerobically grown E. coli cells.

MATERIALS AND METHODS

E. coli strain WG1146 a ser A auxotrope used in this work was supplied by Dr. W. B. Dempsey, Veterans Administration Hospital, Dallas, TX. The medium and growth conditions were the same as described earlier (10,11) except for the substitution of 100 mg/L $MgCl_2 \cdot 12\ H_2O$ for the $MgSO_4$, 30.0 mg/L L-[sulfane-^{34}S] thiocystine for the cystine, 800 mg/L DL-serine for the serine and no glycine or methionine. The labeled thiocysteine was prepared and characterized as previously described (10).

Analysis of the sulfide released from acidified whole cells. Cells (100-200 mg) were removed from the growth medium by centrifugation (5000 x g, 10 min) at the end of log phase growth and washed 2X at 3°C with 5 ml of 0.9% sodium chloride. The cell pellet was then transferred in 0.5 ml of 0.9% sodium chloride to a one-dram vial containing a small tube for the

sodium hydroxide absorption solution and a magnetic stirrer. The height and shape of the absorption tube was such that it could be stirred along with the cell suspension without any physical mixing of the two solutions. The cap of the vial, modified with a teflon-lined septum, was placed on the vial and the vial was flushed with nitrogen gas for 10 min at room temperature. At the end of this period, 0.1 ml of an oxygen-free solution of 0.1 M sodium hydroxide was added to the central absorption tube followed by 0.2 ml of oxygen-free 1 M HCl added to the cell suspension. Both the cell suspension and absorption solutions were then stirred during the diffusion of the hydrogen sulfide. At the completion of the diffusional transfer of the hydrogen sulfide, the hydrogen sulfide trapped in the absorption solution was measured by a modified method of Fogo and Popowsky (12) as described by Lovenberg et al. (13).

Measurement of the sulfur-34 in the released hydrogen sulfide. Alternatively, the sodium hydroxide absorption solution described above was mixed under nitrogen with 0.4 ml of 0.6 M sodium bicarbonate, 0.4 ml methanol and 10 µl of benzyl chloride. After warming at 60°C for 1 hr, the resulting dibenzylsulfide was extracted from the reaction mixture with methylene chloride. Gas chromatography-mass spectrometry (GC-MS) analysis of the molecular ion at m/z 214 in the 70 eV mass spectra of the dibenzylsulfide was used to measure the sulfur-34 abundance in the released hydrogen sulfide.

Measurement of the sulfur-34 abundance in the cellular cysteine and methionine. The sulfur-34 abundance in the bound cysteine and methionine present in the cells was measured by GC-MS of the S-methyl-N-trifluoroacetyl-n-butyl ester of the cysteine and the N-trifluoroacetyl-n-butyl ester derivative of the methionine as previously described (14).

RESULTS

Fig. 1 shows the time course for the release of hydrogen sulfide from an acidified solution of sodium sulfide containing 27 nmoles of sulfide and from an acidified suspension of E. coli cells containing 12 mg of protein (biuret). Both samples show about the same rate of release of hydrogen sulfide which is complete in about 30 min. The efficiency of trapping observed, about 85% in this experiment, was found to be dependent upon the degree of anaerobiosis and the amount of hydrogen sulfide assayed. For the analysis of 30 nmolar amounts of hydrogen sulfide, no hydrogen sulfide could be found in the absorption solution unless the cells were first flushed with nitrogen. This effect is clearly due to the oxidation of the hydrogen sulfide by the oxygen present in the absorption flask (15). Since this oxidation reaction will not affect the sulfur-34 abundance in the hydrogen sulfide released from the cells, the important consideration is to have the O_2 concentration in the absorption cell low enough so that most of the hydrogen sulfide can be trapped and assayed for sulfur-34. No hydrogen sulfide was measured in controls (no added sulfide) or from 0.5 ml of a

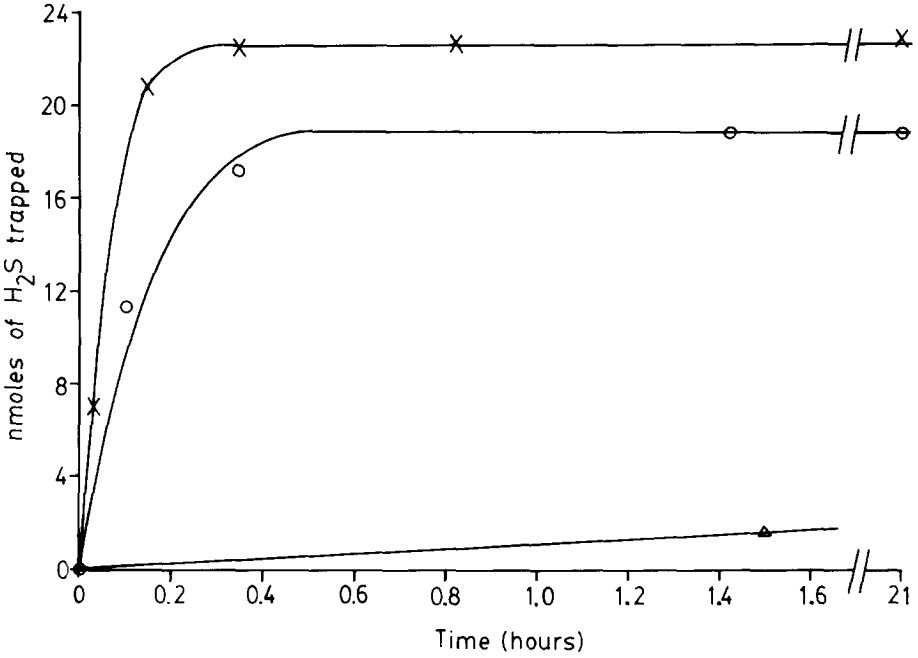

Fig. 1. Time course for the release of hydrogen sulfide from acidified solutions containing (X) 27 nmoles sodium sulfide and (O) a suspension of E. coli cells (12 mg protein). (Δ) represents H_2S released from a non-acidified suspension by E. coli cells (12 mg protein).

0.16 M solution of cysteine. Nonacidified cell suspensions gave only a small release of hydrogen sulfide as shown in Fig. 1.

Measurement of the sulfur-34 in the hydrogen sulfide released from the acidified cells showed it to contain 30.7 ± 1.2 atom % sulfur-34. The cysteine and methionine in the cells contained 30.6 ± 0.6 atom % and 30.6 ± 0.7 atom % sulfur-34, respectively. Methionine isolated from the cells showed only the natural abundance of sulfur-34 when it was added to the growth medium at 0.4 mM.

DISCUSSION

Before the isotopic data from this experiment could be interpreted it had to be firmly established that the hydrogen sulfide released from the acidified E. coli cells actually originated from the sulfide present in the iron-sulfur proteins. This was established in the following way. First, the rate of release of the hydrogen sulfide from the cells was almost as

fast as the rate of released hydrogen sulfide from an aqueous solution of sodium sulfide (Fig. 1). This is consistent with the observed rapid breakdown of iron-sulfur proteins when they are assayed for sulfide under the standard acidic conditions (13). Second, the release of hydrogen sulfide from the cells stopped completely after about 30 min. Based on the amount of protein present in the cells and assuming the protein contains 1% cysteine/cystine, the total amount of hydrogen sulfide released from the cells represented less than 2% of the total cellular cysteine/cystine. This indicated that the bulk of the cysteine was not supplying the hydrogen sulfide and is consistent with many earlier observations which clearly demonstrated that cysteine and acid "labile" sulfide of iron-sulfur proteins represent separate sulfur classes (12,16). Finally, there is substantial evidence for the occurrence of a large number of iron-sulfur proteins in aerobically grown E. coli cells. These include ferredoxin (17), succinate dehydrogenase (18), membranal DPNH dehydrogenase (19), and glutamate synthase (20).

Considering the above evidence, we must conclude that the major portion of the hydrogen sulfide released from the acidified cells originates from the entire assemblage of the iron-sulfur proteins present in the bacteria. We can now assume that the label in this sulfide, 30.7 atom % sulfur-34, represents the label found in the sulfide of the iron-sulfur proteins in vivo.

This isotopic enrichment of the acid "labile" sulfide must now be compared with the sulfur-34 abundance found in the cellular H_2S, cysteine and methionine in order to determine its biosynthetic origin. Previous work has shown that E. coli cells metabolize [sulfane-^{34}S] thiocystine in such a way that the hydrogen sulfide present in vivo can be labeled to the extent of 45-50% with sulfur-34 (10). Considering that the cysteine and methionine both contained an abundance of sulfur-34 of 30.6%, it is clear that the sulfide in the iron-sulfur proteins had to originate from an organic sulfur source and not from the cellular hydrogen sulfide. In addition, since growth with unlabeled methionine changed only the sulfur-34 abundance in the

methionine it is clear methionine is not the source of this sulfur. We can, therefore, conclude that the sulfur used for the biosynthesis of the bulk of the iron-sulfur proteins in E. coli comes from an organic source that is not in equilibrium with the cellular sulfide. A logical choice of this organic sulfur would be cysteine.

That the free sulfide in the cells, i.e. that which is used for cysteine biosynthesis, can be maintained in isotopic disequilibrium from the iron-sulfur protein sulfide is, at first, unexpected. However, if one considers the very slow exchange observed between sulfide ion and iron-sulfur centers at neutral pH's (21) as well as the small (~8%) exchange observed in vivo between sulfide and cysteine (22), it is clear that this disequilibrium can exist.

The results reported in this paper are consistent with the work of Taniguchi and Kimura (7) who concluded that sulfur originating from cysteine by the combined action of a transaminase and 3-mercaptopyruvate sulfurtransferase was involved in the formation of iron-sulfur proteins.

The proof of the involvement of this enzyme in the formation of iron-sulfur proteins would give a rational explanation for its widespread occurrence (23,24). It would also establish a functional role for this enzyme in cellular metabolism for which there is, at present, none.

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