Transsulfuration in Archaeabacteria

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The transfer of sulfur from methionine to cysteine in the archaea bacteria Sulfoborus acidocaldarius and Halobacterium marismortui was studied by feeding [34S]-labeled methionine to cells and measuring the incorporation of [34S] into protein-bound cellular cysteine and methionine by mass spectrometry. It was found that, as are eucaryotes, both of these archaea bacteria were able to convert the sulfur of methionine to cysteine.

Transsulfuration, the transfer of sulfur from cysteine to homocysteine via cystathionine, represents an important series of reactions in the metabolism of reduced sulfur (3). At present, three patterns of transsulfuration, each using a different series of reactions, have been described for different organisms. In the mammalian type of transsulfuration, homocysteine derived from methionine is converted unidirectionally to cysteine, whereas in bacteria and plants (6) cysteine is converted to homocysteine which is then converted into methionine. In each case, the exact flow of the intermediate is controlled largely by the specificity of the cystathionase present in the different cells (3).

We now report that, as do mammals, members of the archaea bacteria have been found to readily convert the sulfur of methionine to cysteine. This finding is based on the measurement of the incorporation of [34S] into protein-bound cysteine by cells grown in the presence of [34S]-labeled methionine.

Cells of different archaea bacteria were grown on the media indicated in Table 1 to which the indicated amounts of labeled methionine were added. The methionine contained the indicated amounts (atoms percent) of molecules with a C3H3[34S] unit (Table 1) and was prepared as previously described (12). At the end of growth, the cells were removed from the medium by centrifugation and washed with water, and the protein-bound cysteine and methionine were isolated and converted to the S-methyl n-butyl trifluoroacetyl derivative for the cysteine and to the n-butyl trifluoroacetyl derivative for the methionine as previously described (8). Gas chromatographic-mass spectrometric analysis of these derivatives was used to measure the isotopic incorporation into each of these molecules. Because the procedure used (8) involved dialysis of cell pellets solubilized in 6 M urea, all of the non-protein-bound amino acids were removed before acid hydrolysis was performed. The [34S] content of the cysteine was calculated from the isotopic enrichment of either the molecular ion at m/z 287 or the m/z 61 fragment ion [CH3SCH2]1+ as previously described (8). Measurement of the [34S] enrichment in the methionine was made more difficult because of the presence in some of the methionine of a trideuterated methyl group resulting from the direct incorporation of the doubly labeled methionine into cellular protein without cleavage of the methyl-sulfur bond of the methionine. The extent of incorporation of the intact labeled methionine can be calculated from the relative intensities of the isotope-containing ion at 5 m/z higher than the nonlabeled ion. Thus, the extent of incorporation of the intact methionine can be measured from either the [C3H3SCH2]1+ fragment ion ([intensity of m/z 66]/[intensity of m/z 61 + intensity of m/z 66]) or the molecular ion ([intensity of m/z 306]/[intensity of m/z 301 + intensity of m/z 306]). In addition, the metabolic cleavage of the methionine to [34S] homocysteine, followed by its subsequent remethylation, would produce methionine containing only [34S]. The extent of this reaction can be calculated from the relative intensities of the ion at 2 m/z higher than the nonlabeled ion after the ion intensity is corrected for the natural occurrence of [34S] (8). Thus, the total incorporation of [34S] from the labeled methionine into protein-bound methionine will be the sum of the methionine molecules containing either a CH3[34S] unit or a C3H3[34S] unit.

For Halobacterium marismortui, 89% of the protein-bound methionine and 87% of the protein-bound cysteine were found to be derived from the labeled methionine (Table 1). The same high atoms percent excess of [34S] measured in both the cysteine and the methionine indicates that essentially all of the cysteine sulfur was derived from the methionine. In addition, from the atoms percent of the methionine containing a CH3[34S] group and a C3H3[34S] group, it can be concluded that 59% of the methionine must have been resynthesized from homocysteine that was derived from the labeled methionine. The fact that the [34S] incorporated in both the cysteine and methionine was less than 100% can be explained by the uptake by the cells of a small amount of unlabeled cysteine and cystine and methionine present in the peptone of the medium.

The derivation of nearly all of the cysteine and methionine sulfur from the fed methionine by H. marismortui is to be compared with the incorporation observed in Sulfoborus acidocaldarius grown on yeast extract plus labeled methionine. In these cells, 93% of the protein-bound methionine was found to be derived from the fed methionine, whereas only 38% of the cysteine was derived from the methionine sulfur (Table 1). Thus, an additional source of sulfur must be present to account for the reduced amount of [34S] incorporated into the cysteine. This sulfur would have to arise from unlabeled cysteine which is either present in the medium or newly biosynthesized in the cells from an unlabeled sulfur source. Since these cells did not incorporate [34S]2− into the cysteine or methionine produced by the cells, the most likely explanation is that the unlabeled cysteine or cystine in the rich medium is being used as a source of cysteine. (The statement regarding the lack of [34S]2− incorporation into...
cysteine and methionine is based on the lack of incorporation of $^{34}$SO$_2^{2-}$ into caldariellaquinoine, a sulfur-containing metabolite of *Sulfolobus* spp., which derives its sulfur from cysteine and/or methionine ([13]). As in the halobacterium, about half of the methionine incorporated by *S. acidocaldarius* was broken down to homocysteine and remethylated to methionine.

In comparison, when *S. acidocaldarius* cells were grown with tryptone as the carbon source, the labeled methionine was found to be incorporated into the cellular methionine to the extent of 83%, 59% of which was metabolized to homocysteine and remethylated to methionine by the cells. In this experiment, however, only 26% of the cysteine was derived from the methionine sulfur, as compared with 38% when the cells were grown with yeast extract. This depression of incorporation into both the cysteine and methionine most likely results from the greater amount of cysteine and methionine in the tryptone than in the yeast extract (7). The ability of *S. acidocaldarius* to derive more of its cysteine sulfur from the medium than does *H. marismortui* may reflect a species-specific difference in the cell’s ability to take up cysteine from the medium.

In contrast to the example described above, in which transsulfuration from methionine to cysteine was readily determined, establishing the occurrence of transsulfuration in the methanogenic archaeabacteria by using the methods described has a number of problems. The major problem is that all of the media used for the growth of methanogens contain high levels of cysteine and/or cysteine and sulfide in order to maintain the medium in a strongly reducing condition (1). Even a recently developed medium with titanium (III) citrate as a substitute for sulfide still contains cysteine (10). One would expect this cysteine to be taken up by the cells and to block the conversion of the methionine sulfur to cysteine sulfur. When an attempt was made to grow *Methanobacterium formicicum* in a cysteine-free titanium(III) citrate medium (10) with methionine as the sole sulfur source, growth occurred only when sulfide was added to the medium. Under these growth conditions, however, none of the methionine sulfur was found to be incorporated into the protein-bound methionine, making it impossible to determine whether transsulfuration had occurred.

The ready transfer of methionine sulfur to cysteine was previously believed to occur only in eucaryotes. Its demonstration in the archaeabacteria adds further support to the closer association of the domain Archaea with the domain Eucarya than with the domain Bacteria as recently outlined by Woese et al (9). One must be careful about these conclusions, however, since the original work of Delavier-Klutchko and Flavin in 1965 (2), which first demonstrated the inability of bacteria to convert methionine to cysteine, was only with two closely related bacteria, *Escherichia coli* and *Salmonella typhimurium*, and has been extended to only one other bacterium, *Synechococcus leopoldiensis* (5). Since these examples represent such a small sample of all the known eubacteria, and since the archaeabacteria can readily convert methionine sulfur to cysteine, one must seriously consider that this type of transsulfuration may in fact occur in at least some eubacteria.

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REFERENCES


