EVIDENCE FOR THIOSULFATE FORMATION DURING SULFITE REDUCTION BY DESULFOVIBRIO VULGARIS.

John E. Findley and J. M. Akagi

Department of Microbiology, University of Kansas
Lawrence, Kansas 66044

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The sulfate-reducing bacterium, Desulfovibrio vulgaris, reduces sulfite to sulfide which accumulates as the end product of sulfate metabolism. When the course of the reduction was followed by analyzing reaction mixtures for sulfide and thiosulfate, the latter compound was observed to accumulate and eventually disappear. However, the sulfide concentration gradually increased and its accumulation was concomitant with thiosulfate disappearance. The results indicate that thiosulfate is one of the intermediate compounds which is formed during dissimilatory sulfite reduction.

Sulfate-reducing bacteria reduce inorganic sulfate to sulfite through the intermediary formation of adenosine-5'-phosphosulfate (APS) (Peck, 1959; Ishimoto and Fujimoto, 1959). The subsequent reduction of APS to AMP and sulfite is catalyzed by APS-reductase (Peck, 1961). The terminal steps of sulfite reduction to sulfide has not been elucidated; however, Kobayashi et al. (1969) presented evidence for the formation of trithionate by Desulfovibrio vulgaris during sulfite reduction. During our studies on the sulfite reductase system of D. vulgaris, we isolated a "thiosulfate-forming system" which reductively transformed bisulfite to thiosulfate (Suh et al. 1968; Suh and Akagi, 1969). Since the possibility exists that thiosulfate is an intermediate between bisulfite and sulfide, this study was conducted to demonstrate the formation of thiosulfate during bisulfite reduction.

MATERIALS AND METHODS

The cultivation, harvesting and preparation of cell-free extracts

of D. vulgaris, strain 8303, was previously described (Akagi and Campbell, 1962). Reactions were conducted in a conventional Warburg apparatus using standard manometric methods. Thiosulfate was determined according to Sorbo (1957) and inorganic sulfide was estimated by the method of Fogo and Popowski (1949). Trithionate was synthesized by the method of Willstatter (1903). Protein was measured according to Lowry et al. (1951). Thin layer chromatography for sulfur compounds was conducted on 20 x 20 cm glass plates coated with Silica Gel. The developing solvent consisted of isopropanol, acetone and water (5:2:3), containing 2% potassium acetate and the detection of sulfur compounds was accomplished by spraying the plates with 0.1% $AgNO_3$ solution.

RESULTS AND DISCUSSION

Evidence for the direct reduction of sulfite to sulfide has never been obtained from studies on sulfate-reducing bacteria. The formation of highly labile intermediates between sulfite and sulfide has been given some considerations; however, the alternate possibility, i.e., formation of stable intermediates, also exists. When crude extracts of D. vulgaris were incubated with bisulfite, under a hydrogen atmosphere, the formation of both thiosulfate and sulfide were detected.

Fig. 1 shows that with a relatively low protein concentration the level of thiosulfate rose rapidly and subsequently disappeared. Concomitantly, the sulfide level increased continuously until the only product of bisulfite reduction was sulfide. Increasing the extract concentration to 10 mg, under identical conditions, resulted in the reaction being completed in 20 minutes and the only detected product was sulfide. Attempts to detect thiosulfate at shorter intervals of time were unsuccessful. To confirm the intermediary formation of thiosulfate at higher concentrations, the level of bisulfite was increased to 10 μ moles. Table 1

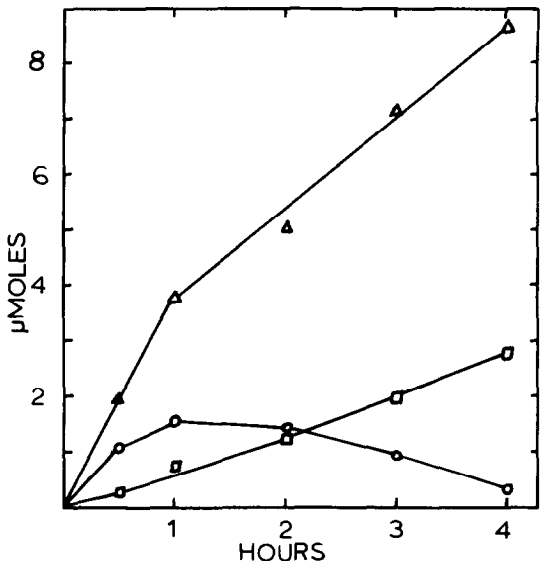

Fig. 1. Formation of Thiosulfate During Bisulfite Reduction. Reactions were conducted in duplicate Warburg flasks. Each flask contained in μmoles: potassium phosphate buffer, pH 6.0, 100; methyl viologen, 1.0; crude extract, 3.2 mg; $NaHSO_3$,5 and water in a total volume of 1.0 ml. Center well contained 0.1 ml of 20% $CdCl_2$. One set of flasks contained 0.1 ml of 20 NH_3PO_4 which was tipped into the reaction mixture to volatilize dissolved sulfide. The other set of flasks did not contain H_3PO_4 and was analyzed directly for thiosulfate. Gas phase, H_2; temperature, 30 C. The symbols are represented as follows: triangles, H_2 utilization; circles, thiosulfate formation; squares, sulfide formation.

TABLE 1 Effect of Time on Thiosulfate and Sulfide Formation

Time (minutes)	μMoles Thiosulfate	μMoles Sulfide	μMoles H_2 utilized
5	0.9	0.2	2.5
30	2.9	3.0	12.0
60	0.3	5.0	16.5

Conditions identical to those described for Fig. 1 except that concentrations of $NaHSO_3$ and crude extract were 10 μmoles and 10 mg respectively.

shows that with 10 mg protein, increasing the substrate concentration resulted in our detecting thiosulfate during bisulfite reduction. Under these conditions the length of time for this reaction was 60 minutes; however, the synthesis and utilization of thiosulfate was again evident.

Because a relatively low extract or high bisulfite concentration resulted in thiosulfate detection from reaction mixtures, and since thiosulfate formation was quite rapid, the inhibition of thiosulfate-reductase by bisulfite, thiosulfate or both cannot be overlooked. The inhibition of thiosulfate-reductase by sulfite was reported for yeast (Kaji and McElroy, 1959) and for Escherichia coli (Artman, 1956). The inhibitory effect of thiosulfate may explain the relatively long reaction time observed with low extract concentration (Fig. 1). The rate of thiosulfate reduction, as seen in Table 2, is greater at lower thiosulfate concentrations than at higher levels. Thus, under conditions

TABLE 2 Effect of Thiosulfate Concentration on Sulfide Formation

Thiosulfate concentration (μmoles)	Sulfide formation (μmoles)
1.0	2.0
2.0	1.7
3.0	1.0
4.0	0.7

Reaction mixtures contained in μmoles: potassium phosphate buffer, pH 6.0, 100; methyl viologen, 1.0; $Na_2S_2O_3$, as indicated; crude extract, 10 mg and H_2O in a total volume of 1.0 ml. Center well contained 0.1 ml of 20% $CdCl_2$; one side arm contained 0.1 ml of 20N H_3PO_4. Gas phase, H_2; temperature, 30 C. After 30 minutes incubation, H_3PO_4 was tipped into the main compartment and after shaking for an additional 5 minutes, the filter paper in the center well was analyzed for sulfide.

where thiosulfate formation is relatively rapid, the reduction of this compound may be the rate-limiting step of sulfite reduction.

Kobayashi et al. (1969) reported that D. vulgaris extracts formed trithionate as a precursor to thiosulfate. Under the conditions employed in this study we were unable to detect this compound. Our efforts to identify trithionate involved the developing of reaction mixtures, at various time intervals, on thin layer chromatography plates. Comparing the samples with thiosulfate, trithionate and tetrathionate the only spot observed with the reaction mixtures was that corresponding to thiosulfate. Another possibility for a sulfur compound which may be a precursor to thiosulfate is dithionite. Postgate (1956) observed that extracts of D. vulgaris reduced dithionite with molecular hydrogen. We also observed this reduction and if the reaction sequence of bisulfite reduction proceeds through dithionite and thiosulfate, this pathway would be similar to that proposed by Woolfolk (1962) for sulfite reduction by Micrococcus lactilyticus.

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REFERENCES

1. Akagi, J. M. and Campbell, L. L., J. Bacteriol. 84, 1194 (1962).
2. Artman, M., J. Gen. Microbiol. 14, 315 (1956).
3. Fogo, J. K. and Popowski, M., Anal. Chem. 21, 732 (1949).
4. Ishimoto, M. and Fujimoto, D., Proc. Japan Acad. 35, 243 (1959).
5. Kaji, A. and McElroy, W. D., J. Bacteriol. 77, 630 (1959).
6. Kobayashi, K., Tachibana, S. and Ishimoto, M., J. Biochem. (Tokyo) 65, 155 (1969).

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem. 193, 265 (1951).

8. Peck, H. D., Proc. Natl. Acad. Sci. U. S. 45, 701 (1959).

9. Peck, H. D., Biochim. Biophys. Acta 49, 621 (1961).

10. Postgate, J. R., J. Gen. Microbiol. 14, 545 (1956).

11. Sorbo, B., Biochim. Biophys. Acta 23, 412 (1957).

12. Suh, B., Nakatsukasa, W. and Akagi, J. M., Bacteriol. Proc. p. 133 (1968).

13. Suh, B. and Akagi, J. M., J. Bacteriol. (In press).

14. Willstatter, R., Ber. 36, 1831 (1903).

15. Woolfolk, C. A., J. Bacteriol. 84, 659 (1962).