PURIFICATION OF A UNIQUE BISULFITE-REDUCING ENZYME FROM DESULFOVIBRIO VULGARIS

H. L. Drake and J. M. Akagi
Department of Microbiology
University of Kansas
Lawrence, Kansas 66045

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SUMMARY: An enzyme which catalyzes the reduction of bisulfite to sulfide and thiosulfate was purified from extracts of the sulfate-reducing bacterium, Desulfovibrio vulgaris. Trithionate was not a product of this reaction nor was it or thiosulfate reduced by the enzyme. High substrate concentrations inhibited sulfide but not thiosulfate formation. The enzyme was named bisulfite reductase II to distinguish it from bisulfite reductase which reduces bisulfite to trithionate.

In biological systems the reduction of sulfite to sulfide occurs by either the assimilatory or dissimilatory pathway. Work with plant (1,2), yeast (3,4) and bacterial (5-7) assimilatory sulfite reductase have shown that sulfite was stoichiometrically reduced to sulfide without the formation of any detectable intermediates. Sulfate-reducing bacteria reduce bisulfite to sulfide by a dissimilatory pathway containing trithionate and thiosulfate as intermediate compounds (8,9). It was reported that these organisms also contain an assimilatory sulfite reductase, however, the dissimilatory route accounted for over 90% of the sulfite-reducing activity in crude extracts (6). The enzyme catalyzing the first step of the dissimilatory pathway is bisulfite reductase which reduces bisulfite to trithionate (10,11). This enzyme has been purified from several sources and some of its physical and chemical properties were characterized (10-14).

This paper describes the purification and some properties of a bisulfite-reducing enzyme from Desulfovibrio vulgaris which is catalytically distinct from the assimilatory and dissimilatory (bi) sulfite reductases.

METHODS

Desulfovibrio vulgaris, NCIB 8303, was cultivated, harvested and disrupted as previously described (15). Seven hundred ml of extract containing ap-

proximately 30 mg/ml protein were fractionated with ammonium sulfate. The fraction precipitating 0.5 and 0.7 saturation was dissolved in a minimum volume of 0.05 M buffer, pH 7.0 (potassium phosphate buffer was used throughout the purification procedure) and dialyzed against 10^{-3} M buffer, pH 7.0. The fraction was passed through a 2.5 x 5 cm column of Amberlite CG-50, type 2 to remove residual cytochrome c_3 and the unadsorbed fraction was applied to a 4 x 25 cm diethylaminoethyl (DEAE)-cellulose (chloride) column. The column was washed successively with 400 ml of 0.01 M buffer, pH 7.5 and 600 ml 0.05 M buffer, pH 7.5. The bisulfite reductase II activity was eluted with 0.1 M buffer, pH 7.5, dialyzed against 10^{-3} M buffer, pH 7.0 and lyophilized. The material was dissolved in a small volume of 0.02 M buffer, pH 7.5 and passed through a 2.5 x 30 cm Sephadex G-100 column equilibrated with the same buffer. The second peak (yellowish-brown) contained the bisulfite reductase II activity and the tubes containing this fraction were pooled, dialyzed and lyophilized. The material was dissolved in buffer and passed through a 2.5 x 30 cm Sephadex G-200 column equilibrated with 0.02 M buffer, pH 7.5. The major peak eluting from this column was collected, dialyzed and lyophilized. At this stage the enzyme preparation contained 4 protein bands as judged by analytical polyacrylamide gel electrophoresis. The final purification step was accomplished by preparative gel electrophoresis.

Particulate hydrogenase was prepared as described previously (9). Thiosulfate was measured according to Sorbo (16) and trithionate was estimated by the method of Kelly et al. (17). Sulfide was analyzed according to Fogo and Popowski (18). Thiosulfate formation was confirmed by paper chromatography using a solvent phase of pyridine:n-propanol:water (7:10:10). Sodium bisulfite solutions were freshly prepared in 10^{-3} M disodium ethylenediaminetetraacetate. Trithionate was synthesized as described earlier (19). Protein was determined according to Lowry et al. (20). Absorption spectra were obtained with a Cary 14 recording spectrophotometer. Molecular weight determination was performed by gel filtration on Sephadex G-200 according to Whitaker (21).

RESULTS AND DISCUSSION

The purified enzyme was brown in color and by disc gel electrophoresis a major and minor band was observed. The minor band migrated toward the anode

Table 1. Effect of enzyme concentration on products formation

Protein (mg)	µmole Thiosulfate(a)	Sulfide(b)	Ratio a/b
0.021	0.14	0.33	0.42
0.042	0.20	0.48	0.42
0.084	0.30	0.78	0.39
0.126	0.39	0.95	0.41

Reaction mixture contained in µmoles: potassium phosphate buffer, pH 6.0, 100; methyl viologen, 1.0; $NaHSO_3$, 10; hydrogenase, 0.25 mg and enzyme in a total volume of 1.1 ml. Center well contained 0.1 ml of 20% $CdCl_2$; temperature, 30C, time, 60 minutes, gas phase, H_2.

slightly faster than the major band. The molecular weight of the enzyme was estimated to be 50,000 daltons.

Table 1 shows that both sulfide and thiosulfate formation increased with increasing enzyme concentration. Thiosulfate formation was confirmed by paper chromatography; trithionate was not detected. These data are in contrast with those reported for assimilatory sulfite reductase, which forms sulfide as the sole product (1,3,7), and dissimilatory bisulfite reductase which forms trithionate as its major product (10,19,22).

Fig. 1 shows the effect of the hydrogen ion concentration on sulfide and thiosulfate formation. A pH of 6.0 is seen to be the optimum for the catalytic activity. Since sulfite exists in the protonated form as bisulfite, under these conditions, we conclude that the ionic species acted upon by the enzyme is bisulfite. Trithionate or thiosulfate was not reduced by this enzyme. To the best of our knowledge all assimilatory sulfite reductases have reported pH optima of 7.0 or higher. On the basis of products formation and pH requirement for optimum activity, we designated this enzyme as bisulfite reductase II. This distinguishes it from the dissimilatory bisulfite reductase which has been studied by earlier investigators (10-14,22).

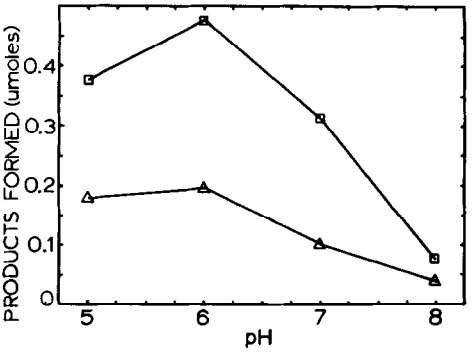

Fig. 1 Effect of pH on products formation. Reaction mixture and conditions were identical to those described under Table 1. Enzyme concentration was 0.09 mg.

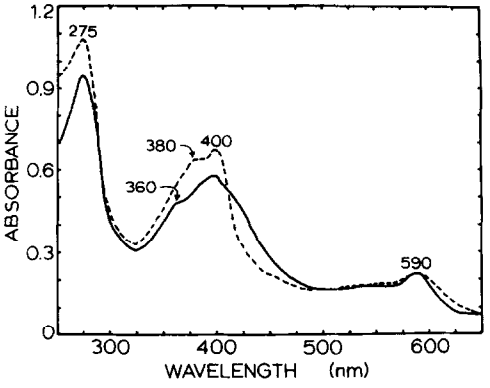

Fig. 2 Absorption spectra of bisulfite reductase II. Each cuvette contained in 1 ml of 0.01 M potassium phosphate buffer, pH 6.0, 0.1 mg enzyme. Solid line represents oxidized form. Broken lines represent 0.1 mg enzyme plus 1 mg $NaBH_4$.

Fig. 2 shows the absorption spectra of bisulfite reductase II. The addition of $NaHSO_3$, KCN or CO did not show any spectral changes. Reduction with $NaBH_4$ resulted in increased absorption at 400 and 275 nm while the 360 nm shoulder shifted to 380 nm. The peak maxima at 590 and 400 nm closely parallel those found in spinach (1), yeast (3) and bacterial (6,23,24) assimilatory sulfite reductases.

Increasing the substrate concentration resulted in an inhibition of sulfide formation while thiosulfate formation was unaffected (Fig. 3). Two

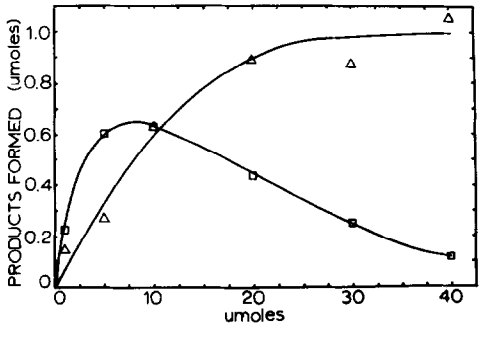

Fig. 3 Effect of bisulfite concentration on products formed by bisulfite reductase II. Conditions were identical to those described under Table 1.

possible explanations are presented to account for these results. One possibility is that bisulfite is reduced to sulfide and thiosulfate through two different routes and its reduction to sulfide is inhibited by high bisulfite concentration while the pathway to thiosulfate is not. Another explanation is that bisulfite is reduced to sulfide according to the following scheme:

$$HSO_3^- \longrightarrow X \longrightarrow S^{2-}$$
$$\downarrow\ HSO_3^-$$
$$S_2O_3^{2-}$$

According to this scheme bisulfite is reduced to sulfide through an intermediate at the oxidation state equivalent to elemental sulfur. At high bisulfite concentrations the reduction of X to sulfide is inhibited and X reacts with (bi) sulfite to form thiosulfate. The intermediate, X, is presumed to be enzyme-bound but still accessible to free substrate. Whether or not this is an enzymatic process has not been determined.

The bisulfite reductase II described in this study appears to be similar to the assimilatory sulfite reductase isolated from D. vulgaris by Lee et al. (6) both in color and in spectral properties. Because of these similarities we have not excluded the possibility that they are the same enzyme. It is interesting to note that Lee et al. (6) assayed their assimilatory sulfite reductase at pH 6.0. These workers also pointed out that the occurrence of another pathway for bisulfite reduction is not surprising if, for some reason, the dissimilatory route involving trithionate and thiosulfate cannot be utilized. If this is the case it seems logical to assume that the substrate for the alternate pathway is the same for the dissimilatory pathway, i.e., bisulfite.

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