FORMATION OF DITHIONITE BY SULFITE REDUCTION WITH *DESULFOVIBRIO VULGARIS* HYDROGENASE AND CYTOCHROME C_3

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Sulfite is reduced to dithionite under hydrogen atmosphere with periplasmic hydrogenase and cytochrome c3 from a sulfate-reducing bacterium *Desulfovibrio vulgaris* Miyazaki K.

KEYWORDS dithionite formation; sulfite reduction; hydrogenase; cytochrome c3; *Desulfovibrio vulgaris*

Desulfovibrio vulgaris, one of the sulfate-reducing bacteria whose biochemical properties have been fairly well studied,[1] has large amounts of highly active hydrogenase and a tetra-heme-containing electron carrier, cytochrome c3. Hydrogenase and cytochrome c3 are located mainly in periplasm and, as a couple, are considered to contribute to the formation of transmembrane electrochemical potential.[2,3] More conventionally, the couple has been postulated to constitute an electron transfer system from molecular hydrogen to various reductases for such as sulfite, thiosulfate, or trithionate ($S_3O_6^{2-}$) all of which are supposed to be intermediary compounds formed during the dissimilatory sulfate reduction to hydrogen sulfide by the sulfate-reducer.[4] The purified sulfite reductase (trivial name desulfoviridin) concomitantly produces trithionate, thiosulfate, and sulfide from sulfite when coupled to hydrogenase and an artificial electron carrier, methyl viologen (MV) dye.[5] In contrast, assimilatory-type NADPH-sulfite reductase from *Escherichia coli*[6] or yeast[7] yields only sulfide as the reduction product *in vitro*. The mechanism of sulfite reduction to sulfide in the *Desulfovibrio* still remains to be elucidated. Recently, the H_2-sulfite reducing system has been reconstructed with hydrogenase, cytochrome c3, ferredoxin I, and desulfoviridin from *D. vulgaris* strain Miyazaki F (MF).[8] But compared with the crude extract the reaction rate was very slow, so some other factor(s) or conditions apparently are required for the reconstitution of a fully active system.

On the other hand, Mayhew *et al.*[9] observed the production of dithionite and SO_2^- by the chemical reaction of (bi)sulfite with MV semiquinone ($MV^{\cdot+}$). They used the hydrogenase-MV couple to generate $MV^{\cdot+}$ and concluded that the following equilibrium between bisulfite and dithionite ions was achieved under their experimental conditions (at pH 6, 25°C);

$$H_2 + 2MV^{2+} \leftrightarrow 2MV^{\cdot+} + 2H^+ \quad (1) \qquad 2MV^{\cdot+} + 2HSO_3^- + 2H^+ \leftrightarrow 2SO_2^- + 2MV^{2+} + 2H_2O \quad (2)$$

$$\downarrow\uparrow$$

$$S_2O_4^{2-}$$

therefore,

$$2HSO_3^- + H_2 \leftrightarrow S_2O_4^{2-} + 2H_2O \quad (3).$$

On our way to reconstituting the sulfite reductase system, we found that, instead of MV, cytochrome c3 in the presence of hydrogenase also reacts with sulfite to form dithionite.

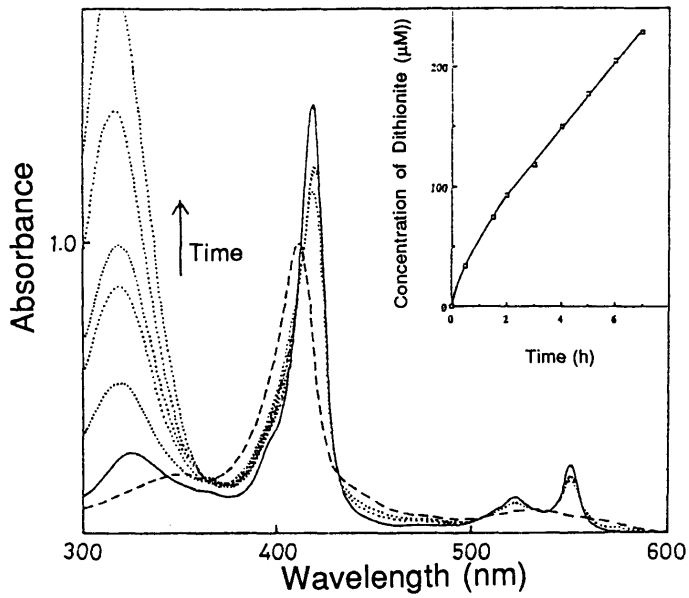

Fig.1. Effect of Sulfite on the Visible and Near UV Spectrum of Reduced Cytochrome c3
To the H_2, hydrogenase, and cytochrome c3 mixture was added $NaHSO_3$, as described under the "MATERIALS AND METHODS."
- - - - - - oxidized cytochrome c3
——— reduced cytochrome c3
····· reduced cytochrome c3 + $NaHSO_3$ (each trace in the direction of the arrow represents; 30, 90, 120, 240 and 300 min after the addition, respectively).
The inset shows the time course of dithionite formation.

Periplasmic cytochrome c3 from *D. vulgaris* strain Miyazaki K (MK) readily gave a fully reduced spectrum (prominent absorption peaks at 552, 523, and 408 nm) under H_2 atmosphere in the presence of catalytic amount of *D. vulgaris* MK periplasmic hydrogenase (Fig. 1). This indicates that the hydrogenase and the cytochrome c3 are functionally related to each other to compose an electron transfer couple from/to hydrogen. When either *D. gigas* periplasmic or *D. vulgaris* MK cytoplasmic hydrogenase was used in place of *D. vulgaris* MK periplasmic hydrogenase, the same results were obtained (data not shown). As shown in Fig. 1., adding an excess of sodium bisulfite to the H_2, hydrogenase, and cytochrome c3 mixture caused a temporary decrease followed by restoration in the visible absorbance of the reduced cytochrome c3, presumably due to oxidation of the cytochrome. In the near UV region, there appeared an absorption maximum around 320 nm and increased its intensity with time and position of the absorption peak approached to 315 nm (attributable to dithionite formation, see below). The difference spectrum of with-minus-without the addition of sulfite gave an absorption maximum at 314 nm. The increase of absorbance at 315 nm was not observed when either one of the components was omitted from the reaction mixture.

The results obtained here coincide very well with those described by Mayhew *et al.*[9] that the addition of sodium bisulfite to H_2-hydrogenase-MV system caused the appearance of a 315 nm-absorption maximum (concluded to be due to the absorption maximum of dithionite based on a difference spectrum and an EPR analyses), except that in our case MV was substituted by the natural electron carrier cytochrome c3. The time course of the dithionite formation is shown in the inset of Fig. 1., on an assumption that, in our case as well as their's, the observed absorption at 315 nm indicates dithionite production from sulfite. Neither thiosulfate, trithionate, nor tetrathionate ($S_4O_6^{2-}$) was detected in the reaction mixture.

Reduced cytochrome c3 is known to reduce sulfur and hydroxylamine.[10] Our finding that reduced cytochrome c3 reacts with sulfite not only adds the sulfite as another entry to possible "substrates" for cytochrome c3 but also should evoke cautious interpretation and designing for the reconstitution of a sulfite reducing system in the sulfate-reducer.

MATERIALS AND METHODS

Hydrogenase and cytochrome c3 were prepared from the periplasmic fraction of *D. vulgaris* MK (IAM 12602) cultivated on a lactate-Polypepton-sulfate medium.[11] The periplasmic fraction was extracted according to Van der Westen *et al.*[12] by pH 9-EDTA treatment of the cell. The extracted periplasmic fraction was brought to 85% saturation with ammonium sulfate. Hydrogenase from the ammonium sulfate precipitated fraction was purified by successive column chromatography on DE32, Sephacryl S300, DEAE-Sepharose, and finally on Sephadex G100. Cytochrome c3 from the 85% saturated ammonium sulfate supernatant of the periplasmic extract was purified according to Ziomek *et al.*[13] by Phenyl-Sepharose, CM-Sephadex, and Sephacryl S300 column chromatography and was lyophilized.

The spectrophotometric study was done in a Thunberg-type cuvette at room temperature. The main compartment of the cuvette (with the light path of 1 cm) contained in 1.5 ml: 50 mM potassium phosphate buffer, pH 6.0, 0.17 μM of hydrogenase, and 2.3 μM of cytochrome c3. The side bulb contained several crystals of $NaHSO_3$ (0.5 - 1 mg). Visibility to the near UV spectrum of oxidized cytochrome c3 was recorded with the absorbance range from 0 to 2 (contribution of the absorption by hydrogenase to the spectrum being negligible under these conditions). The gas phase was then replaced by a few cycles of evacuation-and-flush with H_2 passed through a De-oxo column. After the spectrum of reduced cytochrome c3 was recorded, the main compartment received $NaHSO_3$ from the side bulb and the spectra of the reaction mixture were recorded as before at the indicated time intervals.

The concentration of dithionite in the equilibrium was calculated from the absorbance at 315 nm using the absorption coefficient of 8,000 $M^{-1}cm^{-1}$.[14] The amounts of thiosulfate, trithionate, and tetrathionate after the reaction were determined colorimetrically by a cyanolysis method.[11]

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(Received July 28, 1989)