On the Role of a Cytochrome in the Thiosulfate Reduction by Sulfate-Reducing Bacterium

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(Received January 22, 1955)

In a previous report (1) we have shown that a cell-free extract which was obtained from ground cells of sulfate-reducing bacterium displaved a marked activity of reducing thiosulfate to hydrogen sulfide in the presence of molecular hydrogen. From the extract we could separate hydrogenase and thiosulfate reductase (2) both of which are conceivably involved in the process in question. The mixture of these separated enzymes, however, were found to be totally inactive in cataysing the said reaction. A marked enhancement of the reaction rate was observed when methyl viologen was added to the enzyme mixture, indicating that the dye-stuff acted as an intermediary carrier filling the missing link between the enzyme systems. The course of events occurring in this case was supposed to be as follows:

$$H_2 \cdots \underset{\text{genase}}{\text{Hydro-}} \cdots \underset{\text{carrier}}{\text{Intermediary}} \cdots \underset{\text{reductase}}{\text{Thiosulfate}} \cdots S_2O_3^{--}$$
(Methyl viologen)

Recently we found (3), in confirmation of the report by Postgate (4), that a certain cytochrome (showing absorption maxima at 419 mμ and 553 mμ in reduced state, and at 410 mμ in oxidized state) is contained in the cells of sulfate-reducing bacterium. The present paper deals with the isolation of this cytochrome from bacterial cells and the elucidation of its role as an intermediary carrier, similar to that assigned to methyl viologen in the above scheme, in the mechanism of thiosulfate reduction.

The procedure of isolation of the cytochrome was similar to that for cytochrome *c* (5). Acetone-dried cells of the bacterium were extracted with M/15 phosphate buffer of pH 6.4. On adding acetone to the extract in concentration of 33-66%, there appeared a precipitate which was dissolved in M/150 phosphate buffer of pH 7.0, dialyzed against the same buffer overnight and then passed through the column of ammonium salt of cation-exchange resin, Amberlite IRC-50. While the cytochrome was absor bed on the top

of the column, thiosulfate reductase, hydrogenase and a green pigment (3) as well as the greater part of porteins ran through it without being adsorbed. By elution with a mixture of ammonium chloride and hydroxide, the cytochrome absorbed could be separated in two fractions. Absorption spectra of these two fractions were hardly distinguishable from each other, both showing a maximum at 410 mμ, the Soret band of oxidized form. Compared with the strong absorption at 410 mμ, there was only insignificant absorption at 280 mμ, indicating that the solution of cytochrome contained, if any, only a small amount of proteinous impurities.

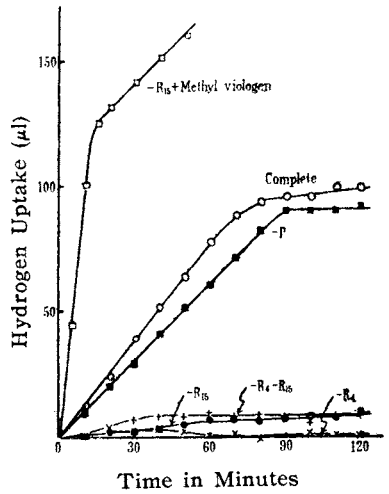

Fig. 1. Reaction between H_2 and thiosulfate (measured by H_2-uptake) caused by reconstructed system of various combinations.

H_2-uptake was measured with Warburg's manometer. Volume of each reaction mixture: 2.25 ml.; temperature: 30° C.; atmosphere: H_2. The center well contained 0.2 ml. of alkali.

The "complete" system was composed of:

(i) 0.5 ml. of suspension of particulate hydrogenase preparation (P) (800 hydrogenase units (2)),

(ii) 0.4 ml. of crude thiosulfate reductase preparation (R4) which was almost free from the cytochrome (70 reductase units (2)),

(iii) 0.75 ml. of the cytochrome preparation (R15) (0.55 cytochrome units (2)),

(iv) 0.2 ml. of 0.2 M phosphate buffer of pH 7.0, containing 6 mg. of egg albumin and 5 μM of $Na_2S_2O_3$.

No hydrogen uptake occurred in the absence of thiosulfate.

The non-absorbed solution containing thiosulfate reductase and hydrogenase showed no activity of reducing thiosulfate in the presence of hydrogen. The same was true when the solution was supplied with an additional quantity of hydrogenase.* A striking increase in the activity of the mixture was observed when the second fraction of the cytochrome was added to the mixture of non-adsorbed solution and hydrogenase preparation. (Fig. 1). Of interest is to note that the first fraction of cytochrome was quite ineffective in accelerating the reaction rate. The effectiveness (as well as the absorption spectrum) of the cytochrome in the second fraction was not modified by boiling the fraction for 3 minutes. This fact the accelerating effect of the fraction is not due to some thermolabile proteinous substances which might have been admixed with the cytochrome in the fraction.

The evidence presented here makes it quite plausible to assumed that the cytochrome obtained in the second fraction is an intermediary carrier, intrinsic to bacterial cells, which functions as a link between the systems of thiosulfate reductase and hydrogenase, just as did methyl viologen in the experiment reported previously. It should be added that the cytochrome in question could not be substituted in its carrier-function by cytochrome *c* from bovine heart muscle (6) or diphosphopyridine nucleotide.

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* Although the original non-adsorbed solution contained a certained amount of hydrogenase, its activity was found to be comparatively less than that of co-existing thiosulfate reductase. The preparation of hydrogenase added was the centrifugate (at 16000×g) from the extract which was obtained from finely ground bacterial cells.

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