PYRUVATE-SUPPORTED ACETYLENE AND SULFATE REDUCTION BY CELL-FREE EXTRACTS OF DESULFOVIBRIO DESULFRICANS

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Summary: Cell-free extracts prepared from a strain of Desulfovibrio desulfricans can reduce acetylene or sulfate, utilizing pyruvate as the electron and ATP source in the presence of methyl viologen, ADP and coenzyme A. Other physiological substances such as (lactate + NAD) and (NADH + ATP) can not reduce acetylene nor sulfate. When acetylene and sulfate are both present as substrates, sulfate represses the acetylene reduction.

A number of sulfate-reducing bacteria have recently been shown to fix nitrogen (1,2), although nitrogenase activity in cell-free extracts has not yet been demonstrated. The bacteria may reduce sulfate and nitrogen both utilizing the electron and ATP arising from the lactate oxidation. It may be then presumed that some relationship between the sulfate and acetylene reduction exists in the bacteria. The present study was attempted to establish a reaction system in which extracts of the bacteria can exhibit both sulfate reduction and nitrogenase activity supported by physiological reductant and energy source.

EXPERIMENTAL

A strain of Desulfovibrio desulfricans capable of fixing nitrogen has been isolated from soil of rice field at Saitama, Japan, and the organism was used in the present study. The taxonomical and some biochemical properties of the organism will be reported elsewhere. The culture medium (pH 7.2) contained 5g Na-lactate, 2g Na_2SO_4, 1g K_2HPO_4, 2g $MgSO_4 \cdot 7H_2O$, 0.1g $CaCl_2 \cdot 2H_2O$, 0.1g Na-ascorbate, 0.1g Na-thioglycolate, 0.2g casamino acids, 6mg $FeSO_4 \cdot 7H_2O$, and each 0.1mg of H_3BO_3, $CoCl_2$, $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot nH_2O$, $ZnSO_4 \cdot 7H_2O$

and $NaMoO_4 \cdot 2H_2O$ in one liter of de-ionized water. The culture was kept at 30°C under a continuous flow of nitrogen. The cells in a logarithmic phase were harvested, washed twice with 30 mM phosphate buffer (pH 7.0) and suspended in the buffer. The cell suspension, after sparged with argon, was subjected to cell disruption in a French pressure cells, and centrifuged at 25,000 x g for 20 min. The dark brown supernatant was used as cell-free extracts. Assays for acetylene and sulfate reduction were carried out in a Warburg flask at 30°C. 1 ml of the gas in the flask was taken with a syringe, and used for the analysis of ethylene in a Hitachi K-53 gas chromatograph equipped with a H_2-flame ionizing detector unit. A stainless steel column packed with β,β'-oxidipropionitrile on 60-80 mesh active alumina was used. The solution in the center well containing 0.2 ml of 20% KOH was used for measurement of hydrogen sulfide according to the method of St. Lorant (3). Pyruvate was determined by the method of Friedemann and Haugen (4). ATP, ADP and AMP were analyzed by the method of Caldwell (5). Protein was determined by the Folin method (6).

RESULTS AND DISCUSSION

Sulfate-reducing bacteria are shown to generate electrons by the pyruvate oxidation to acetyl phosphate (phosphoroclastic reaction) using ferredoxin and coenzyme A as electron carrier and cofactor, respectively (7), and the acetyl phosphate oxidation is considered to be coupled with the generation of ATP from ADP or AMP (8). It may be then expected that extracts of the present sulfate-reducing bacterium can exhibit pyruvate-supported nitrogenase activity, when ferredoxin, coenzyme A and ADP (AMP) were present. As was expected, the extracts exhibited an appreciable nitrogenase activity, as measured by acetylene reduction, when pyruvate, methyl viologen

Table I: Requirements for acetylene or sulfate reduction by cell-free extracts of Desulfovibrio desulfricans

Omission	Activity (% of complete system)	
	Acetylene reduction	Sulfate reduction
None	100	100
Pyruvate	0	0
Methyl viologen	0	0
ADP	0	27
Coenzyme A	39	58

Complete reaction mixture(final volume, 2.0 ml): 15 mg protein of extracts, 60 μmoles K-phosphate(pH 7.0), 10 μmoles $MgCl_2$, 115 mM Na-pyruvate, 2 μmoles methyl viologen, 5 μmoles ADP and 0.13 μmoles coenzyme A. When sulfate reduction was measured, 10 μmoles sulfate was added to the mixture. Gas phase was 5% acetylene-95% argon or argon for acetylene or sulfate reduction, respectively. Reaction was carried out for 45 min, and terminated by injecting 1 ml of 12% CCl_3COOH solution through the stopper with a hypodermic needle.

substituted for ferredoxin (9), coenzyme A and ADP (AMP). When methyl viologen or ADP (AMP) was omitted from the reaction mixture, no acetylene reduction was observed (Table I). The results may indicate that the extracts could reduce acetylene utilizing the electron and ATP generated by the pyruvate oxidation, as described above. Maximum acetylene reduction (2.9 nmoles ethylene formed/mg protein/hr) was observed at 115, 1.0 and 2.5 mM of pyruvate, methyl viologen and ADP, respectively (Fig. 1).

Sulfate reduction also requires both electron and ATP (10). The extracts, however, exhibited sulfate-reducing activity without ADP (0.23 μmoles hydrogen sulfide formed/mg protein/hr) (Table I), although addition of ADP increased the reduction, and maximum activity (3.4-3.7 times that without ADP) was observed at 0.5-2.5 mM ADP. 1 ml (15 mg protein) of the extracts contained 34, 42 and 38 nmoles of ATP, ADP and AMP, respectively. These amounts of adenosine phosphate seemed to generate sufficient amount of ATP for the sulfate reduction, but insufficient for the acetylene reduction.

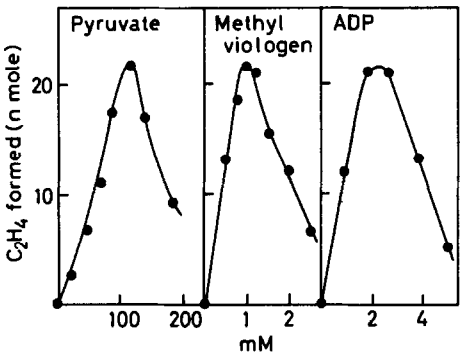

Fig. 1.

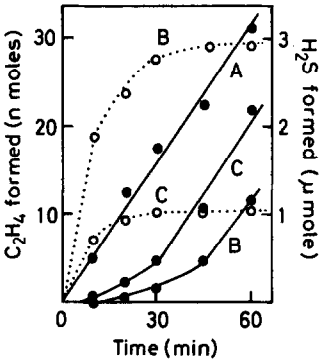

Fig. 2.

Fig. 1. Effects of varing concentrations of pyruvate, methyl viologen and ADP on acetylene reduction. Reaction mixture was identical to that in Table I, except where variations are indicated. Gas phase, 5% acetylene-95% argon. Reaction time, 30 min.

Fig. 2. Effect of sulfate on acetylene reduction. Reaction mixture was identical to that in Table I. Gas phase, 5% acetylene-95% argon. A, control; B, 3 μmoles sulfate; C, 1μmole sulfate. Acetylene reduction, —●— ; Sulfate reduction, ···○··· .

Lactate may be converted to pyruvate in cells, probably, by lactic dehydrogenase (EC 1.1.1.27), although the conversion has not yet been demonstrated in cell-free extracts. When lactate (110 mM) and NAD (1.4 mM) were substituted for pyruvate, neither acetylene nor sulfate reduction was observed. NADH, when added to the extracts at 0.2 mM, was oxidized at a considerable rate (10 nmoles NADH oxidized/mg protein/min), but addition of pyruvate (2.1 mM) resulted in a more rapid oxidation of NADH (four times that without pyruvate), suggesting the presence of lactic dehydrogenase in extracts. When lactate (110 mM) and NAD (0.7 mM) were added to the extracts, NAD was reduced only slowly (1.7 nmoles NADH formed/mg protein/min) and pyruvate was formed at a rate of 5 nmoles/mg protein/min. This amount of pyruvate seemed too small to support the acetylene or sulfate reduction by the extracts. In whole cells, some reactions consuming rapidly NADH may occur, so that pyruvate may be formed in enough amount to support the acetylene or sulfate reduction.

NADH seemed not to act as an electron source for both acetylene and sulfate reduction in cells. NADH (15 mM) was unable to reduce acetylene nor sulfate in extracts, when 8 mM ATP was used as an energy source in the presence of methyl viologen and coenzyme A.

The pyruvate-supported acetylene reduction by the extracts was strongly repressed by sulfate (Fig. 2), and when most amount of sulfate was reduced the acetylene reduction commenced. The repressive effect of acetylene reduction by sulfate may be considered to be due to the competition between the acetylene and sulfate reduction in utilizing electron and ATP, rather than due to the nitrogenase inhibition by sulfate. Whole cells, when incubated in the culture medium under acetylene-argon gas phase, reduced both acetylene and sulfate simultaneously. The cells, however, when incubated in the medium from which sulfate was omitted, could not reduce acetylene. The lactate oxidation to pyruvate or entry of lactate into cells seemed to necessitate the presence of sulfate. The apparently different effects of sulfate on the acetylene reduction observed between in whole cells and extracts are now being examined.

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