PROTON TRANSLOCATION ASSOCIATED WITH SULFITE REDUCTION IN A SULFATE-REDUCING BACTERIUM, *DESULFOVIBRIO VULGARIS*

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1. Introduction

It has long been postulated that sulfate-reducing bacteria conduct anaerobic respiration, conserving energy through oxidative phosphorylation in anaerobic sulfate reduction [1,2]. Experimental results supporting this hypothesis have been reported [1–3]. From chemiosmotic theory [4], electron transport in respiration should be associated with proton translocation across biomembranes. However, proton translocation coupled to electron transport for the reduction of inorganic sulfur compounds has not yet been reported. Here, we demonstrate proton translocation associated with sulfite reduction in a sulfate-reducing bacterium, *Desulfovibrio vulgaris*.

2. Materials and methods

2.1. *Preparation of cell suspensions*

Cells of *Desulfovibrio vulgaris*, strain MK [5], were grown anaerobically in a lactate–ammonium sulfate–Polypepton medium [6] at 37°C for 12–14 h, followed by a refreshment in the same medium and incubation for a further 4 h. Cells were harvested, washed 3 times with 120 mM KCl, and suspended in 120 mM KCl.

2.2. *Measurements of pH changes*

The pH changes of the cell suspensions were measured during oxidant pulses with a combination electrode (type GK2321 C, Radiometer) connected to a pH meter (type PHM 84, Radiometer) [7]. Cell suspension (2 ml, 2–3 mg protein/ml) containing 120 mM KCl, 7.5–60 mM KSCN, and 25 μg carbonic anhydrase/ml (Boehringer-Mannheim) were stirred magnetically and equilibrated under hydrogen gas at 25°C. Solutions with electron acceptors in 120 mM KCl were injected into the cell suspensions after being bubbled with nitrogen gas. The proton/oxidant ratio was corrected for re-entering H^+ [8]. 3,5-Di-*tert*-butyl-4-hydroxylbenzylidenemalononitrile (SF6847, Sumitomo Chemicals) was kindly provided by Dr N. Kamo, Faculty of Pharmaceutical Sciences, Hokkaido University.

3. Results

The extracellular pH of *Desulfovibrio vulgaris* suspensions decreased by the addition of 10 nmol $NaHSO_3$ (fig.1A). The proton conductor 3,5-di-*tert*-butyl-4-hydroxylbenzylidenemalononitrile (SF6847) prevented the decrease of the pH at 10 μM (fig.1B). When the hydrogen over the solution was replaced by nitrogen, the magnitude of the acidification decreased gradually, but it was restored by flushing the nitrogen with hydrogen (fig.2). This behavior indicates that sulfite reduction by the hydrogen gas gave rise to an outward translocation of protons in *D. vulgaris*.

The amount of H^+ translocated/mol sulfite reduced (H^+/SO_3^{2-} ratio) was 12–14 under optimum conditions. Maximum acidification was observed at 1–2 h after the initiation of incubation; while earlier, the baseline drift of pH was too fast to measure the accurate amount of H^+ translocation, the amplitude decreased after the maximum (half-life ~2 h). Thiocyanate ions were not necessary for the acidification by the sulfite pulses, but the presence of SCN^- (≥7.5 mM) enhanced the acidification nearly 2-fold.

The proton conductor-sensitive proton extrusion was also observed with potassium bisulfite, and sodium salts of sulfite, metabisulfite, and dithionite. Proton translocation caused by these compounds was mostly

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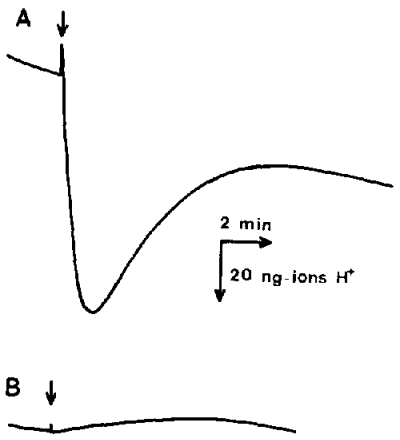

Fig.1. pH changes associated with sulfite reduction in *D. vulgaris* cell suspensions. The cell suspensions contained 2.4 mg cellular protein/ml, 120 mM KCl, 15 mM KSCN, and carbonic anhydrase (25 μg/ml). The gas phase was hydrogen. The extracellular pH was recorded from left to right. The suspensions were pulsed with 10 nmol $NaHSO_3$ (arrows) in the absence (A) and presence (B) of 10 μM SF6847. A downward deflection indicates a decrease in pH.

explained by the presence of sulfite and/or bisulfite ions in the oxidant solutions. No pH change was observed by the addition of sodium sulfate, thiosulfate, trithionate, or NaCl.

A rapid proton extrusion was observed with the addition of air-saturated 120 mM KCl to the anaerobic cell suspensions. It was sensitive to the SF6847 proton conductor. Assuming an oxygen solubility of

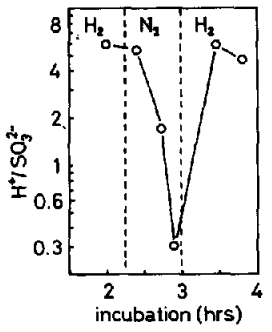

Fig.2. The dependence of H^+ translocation on the hydrogen atmosphere. *D. vulgaris* cells were suspended in 120 mM KCl, 60 mM KSCN, and carbonic anhydrase (25 μg/ml). The gas phase was flushed with hydrogen or nitrogen as indicated. At the indicated times of incubation (○), 10 nmol $NaHSO_3$ were injected into the cell suspension. H^+/SO_3^{2-} ratios (ordinate) were corrected as in [8].

0.48 μg atom/ml, a H^+/O ratio of 9–14 was obtained. The possibility of contamination of aerobic bacteria was eliminated by aerobic culture test, in which no colony was observed on agar plates.

4. Discussion

Chemiosmotic theory predicts that respiratory electron transport is accompanied by translocation of proton [4]. Here, proton translocation coupled to sulfite reduction is shown with cell suspensions of a sulfate reducer, *D. vulgaris*.

Proton extrusion was not observed by the addition of sulfate. It is explained by sulfate being reduced only after its activation by ATP to adenosine phosphosulfate, which is reduced to sulfite [1,2]. The ATP level of the washed, resting cells may be too low to conduct sulfate activation. Sulfite reduction is a key intermediary step in the reduction of sulfate to sulfide; the sulfate to sulfite reduction is endergonic, but the sulfite to sulfide reduction is exergonic [2].

The localization of the enzymes of sulfite reduction has been studied in *Desulfovibrio* spp. [2,9,10]: hydrogenase was found in periplasm and sulfite reductase in the cytoplasmic region. The enzyme location itself could explain the apparent H^+ gradient formation of 2 $H^+/2\ e^-$, if it is considered that hydrogen gas is activated in the periplasm ($H_2 \rightarrow 2\ H^+ + 2\ e^-$), and only electrons are transferred across the cytoplasmic membrane leaving H^+ outside, and sulfite is reduced inside the membrane ($SO_3^{2-} + 6\ H^+ + 6\ e^- \rightarrow S^{2-} + 3\ H_2O$). The observed stoichiometry, 4–5 $H^+/2\ e^-$, derived from 12–14 H^+/mol translocated by the reduction of sulfite to sulfide by 3 pairs of electron, suggests the participation of redox H^+-pump(s) in H^+ translocation associated with sulfite reduction.

Proton translocation has been reported in a strain of *D. desulfuricans* in association with nitrite respiration [11]. Proton translocation by oxygen pulses, which we report here, was not observed with the nitrite-respiring *D. desulfuricans* [11]. The physiological significance of the oxygen-driven proton translocation in a strictly anaerobic *D. vulgaris* remains to be elucidated.

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