Cytochrome cbb_3 of *Thioalkalivibrio* is a Na^+-pumping cytochrome oxidase

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Cytochrome *c* oxidases (Coxs) are the basic energy transducers in the respiratory chain of the majority of aerobic organisms. Coxs studied to date are redox-driven proton-pumping enzymes belonging to one of three subfamilies: A-, B-, and C-type oxidases. The C-type oxidases (cbb_3 cytochromes), which are widespread among pathogenic bacteria, are the least understood. In particular, the proton-pumping machinery of these Coxs has not yet been elucidated despite the availability of X-ray structure information. Here, we report the discovery of the first (to our knowledge) sodium-pumping Cox (Scox), a cbb_3 cytochrome from the extremely alkaliphilic bacterium *Thioalkalivibrio versutus*. This finding offers clues to the previously unknown structure of the ion-pumping channel in the C-type Coxs and provides insight into the functional properties of this enzyme.

cytochrome *c* oxidase | sodium pumping | cbb_3-type oxidase | alkaliphily

The known terminal oxidases according to the structure of their active centers and their phylogenetic relations are subdivided into two superfamilies (1). One is composed of numerous representatives containing a heme-copper binuclear active center (BNC). Oxidases belonging to the other superfamily

$$4\mathrm{cyt}c^{2+} + 4\mathrm{H}^+_{\mathrm{in,chem}} + 4\mathrm{H}^+_{\mathrm{in,pump}} + \mathrm{O}_2 \rightarrow 4\mathrm{cyt}c^{3+} + 4\mathrm{H}^+_{\mathrm{out,pump}} + 2\mathrm{H}_2\mathrm{O}.$$

In A-type Coxs, two H^+ pathways in the main subunit were identified, the so-called D channel, conducting all pumped and part of chemical H^+, and the K channel, conducting most of chemical H^+ (9). In C-type Coxs, only a K-channel analog was found (10). The described catalytic events are accomplished through generation of a transmembrane difference in H^+ potentials ($\Delta\bar{\mu}_{H^+}$), which is used as a convertible membrane-linked biological currency. Microorganisms living in an alkaline environment maintain a nearly neutral cytoplasmic pH (11). This presents a problem for alkaliphiles because it gives rise to an inverted pH gradient that decreases the $\Delta\bar{\mu}_H$

Significance

The majority of aerobic living organisms use oxygen for respiration. The key enzyme, which directly reduces oxygen to water during respiration, is the terminal cytochrome *c* oxidase. It generates a large portion of the utilizable energy provided by the respiratory chain. Accumulation of biologically available energy by means of cytochrome *c* oxidases is believed to be due to the proton-motive force across the mitochondrial or bacterial membrane. Details of this energy conversion are still unclear. Here we report the discovery of a sodium-pumping cytochrome *c* oxidase that converts energy of respiration into sodium-motive force. This finding provides clues to understanding the mechanism of cytochrome *c* oxidase that is not available when applying knowledge of the proton-pumping versions of the enzyme.

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The authors declare no conflict of interest.

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Data deposition: The nucleotide sequences of *Thioalkalivibrio* cbb_3 oxidase have been deposited in the EMBL database (accession no. HE575403.1).

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Electrical membrane potential generation in right-side-out membrane vesicles was monitored by the safranine method (49) or by tetraphenylphosphonium-selective electrode (50) at 25 °C.

H^+ release in intact cells and membrane vesicles in O_2-pulse experiments was assessed by a standard method (51) in 1 mL of anoxic incubation mixture. Respiration of samples was initiated by addition of water (5–20 μL) saturated with air O_2 at 25 °C. The evoked changes in pH in the incubation mixture were estimated by titration with argon-saturated 0.5 mM H_2SO_4.

Respiratory activity was assessed using a Clark-type electrode at 25 °C.

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