

EFFECTS OF THIOCYANATE AND VENTURICIDIN ON RESPIRATION-DRIVEN H^+ TRANSLOCATION IN PARACOCCLUS DENITRIFICANS

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1. A fast-responding O_2 electrode [1] has been used to confirm and extend [2] observations [3,4] of a significant kinetic discrepancy between O_2 reduction and consequent H^+ translocation in " O_2 -pulse" experiments in intact cells of P. denitrificans. The chaotropic SCN^- ion abolishes this discrepancy, and greatly increases the observable $\rightarrow H^+ / O$ ratio, to a value approaching its accepted, true, limiting stoichiometry. The observable H^+ decay rates are very slow, particularly in the absence of SCN^- .

2. The submaximal $\rightarrow H^+ / O$ ratios observed in the absence of SCN^- are essentially independent of the size of the O_2 pulse when this is varied between 4.7 and 47 ng atom, in a manner not easily explained by a delocalised chemiosmotic energy coupling scheme.

3. Osmotically active protoplasts of P. denitrificans do not show a significant kinetic discrepancy between O_2 reduction and H^+ ejection, even in the absence of SCN^- . However, the submaximal $\rightarrow H^+ / O$ ratios observed in the absence of SCN^- are again essentially independent of the size of the O_2 pulse. As in intact cells, the observable H^+ decay rates are extremely slow.

4. The energy transfer inhibitor venturicidin causes a significant increase in the $\rightarrow H^+ / O$ ratio observed in P. denitrificans protoplasts in the absence of SCN^- ; the decay kinetics are also somewhat modified. Nevertheless, the $\rightarrow H^+ / O$ ratio observed in the presence of venturicidin is also independent of the size of the O_2 pulse in the above range. This observation militates further against arguments in which (a) a non-ohmic backflow ("leak") of H^+ from the bulk aqueous phase might alone be the cause of the low $\rightarrow H^+ / O$ ratios observed in the absence of SCN^- , and (b) in which there might be a Δp -dependent change ("redox slip") in the actual $\rightarrow H^+ / O$ ratio.

5. It is concluded that the observable protonmotive

activity of the respiratory chain of P. denitrificans in the absence of SCN^- is directly influenced by the state of the H^+ -ATP synthase in the cytoplasmic membrane of this organism. We are unable to explain the data in terms of a model in which the putative protonmotive force may be acting to affect the $\rightarrow\text{H}^+/\text{O}$ ratio.

6. One possibility, which would conveniently serve to explain these and other [5] data, is that the bulk-to-bulk phase membrane potential set up in response to protonmotive activity is energetically insignificant. Since the apparent membrane potential, as judged by steady-state ion uptake measurements, is insensitive to respiration rate over a wide range [6], one should predict that the kinetics of ion uptake (in a chemiosmotic model) would be similarly insensitive to respiration rate. Such an experimental test might allow one to distinguish the veracity of "localised" [7] and "delocalised" energy coupling models in electron transport phosphorylation [8].

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