

RESPIRATION-DRIVEN  $H^+$  TRANSLOCATION IN PARACOCCLUS DENITRIFICANS; ROLE OF THE 'PERMEANT' ION

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It was shown [1] that the addition of pulses of  $O_2$ , as air-saturated KCl, to weakly-buffered anoxic suspensions of P. denitrificans elicited the vectorial ejection of  $H^+$  into the aqueous phase external to the organisms. Yet, in the absence of valinomycin or  $SCN^-$  the half-time of  $H^+$  translocation was very much greater than the half-time of  $O_2$  reduction, and the extrapolated  $\rightarrow H^+/O$  ratio was also significantly less than that observed when appropriate concentrations of valinomycin or  $SCN^-$  were present. It was proposed that in the absence of such compounds a large bulk-to-bulk phase membrane potential was built up, and that inhibition of this build-up by movement across the membrane of permeant ions allowed the true stoichiometry of  $H^+$  translocation to be observed.

This explanation was challenged by work [2-4] in which the size of the  $O_2$  pulse, in ng atom per vesicle, was varied, in the absence of permeant ions, with little change in the measured  $\rightarrow H^+/O$  ratio, and we have therefore carried out a reinvestigation of the role of compounds such as  $SCN^-$  in  $O_2$ -pulse experiments in P. denitrificans.

Methods. Cells were grown, harvested, washed, incubated, and respiration-driven  $H^+$  translocation measured by conventional means, in a reaction volume of 6 ml.

Results. When the cell concentration was 3 mg dry wt /ml, the following results were obtained: [a] the number of measurable  $H^+$  translocated across the bacterial membrane,  $\Delta H^+$ , increased linearly with the size of the  $O_2$  pulse as this was raised from 4.7 to 47 ng atom, with no 'permeant' ions present, [b] this pattern was unchanged when the cell concentration was changed by a factor of 3 or when NaCl or choline chloride were substituted for KCl in the reaction media, [c] the  $\rightarrow H^+/O$  ratio observed when 100 mM KSCN was present increased from approx 2.5 to approx

7.5, [d] sodium tetraphenylborate at concentrations of 10-60  $\mu\text{M}$  increased the observed  $\rightarrow\text{H}^+/\text{O}$  ratio approximately two-fold. This could not have been caused by electrophoretic movement of tetraphenylborate ions from the inner to the outer bulk aqueous phases since the number of 'extra'  $\text{H}^+$  was greater than the number of free intracellular tetraphenylborate ions, and the number of extra  $\text{H}^+$  seen in the bulk phase at a given tetraphenylborate concentration was independent of the size of the  $\text{O}_2$  pulse between 14 and 42 ng atom.

It is concluded from these observations that in the absence of compounds such as  $\text{SCN}^-$ , protons translocated during electron transport pass directly to membrane-located sinks such as the ATPase via a non-bulk-phase pathway. Mechanistic details have been suggested that can account for the unexpected lack of equilibration of protonic energy between the membrane and the bulk [5].

#### References.

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