Respiration-driven proton translocation in Paracoccus denitrificans: role of the 'permeant' ion

DUNCAN HITCHENS and DOUGLAS B. KELL.
Department of Botany and Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, Wales, U.K.

Scholes & Mitchell (1970) demonstrated that the addition of pulses of O₂ as air-saturated KCl, to weakly-buffered anoxic suspensions of Micrococcus (now Paracoccus) denitrificans elicited the vectorial ejection of H⁺ into the bulk aqueous phase external to the organisms, where they could be detected with a sensitive glass electrode. Yet, in the absence of valinomycin or the SCN⁻ ion, the half-time of H⁺ translocation was very much greater than the half-time of O₂ reduction; the extrapolated →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 transmembrane potential was built up by the translocation of a small fraction of the pumped protons, and that inhibition of this bulk-to-bulk transmembrane potential by the transmembrane electrophoretic co- or counter-transport of 'permeant' ions allowed measurement of the true stoichiometry of respiration-driven H⁺ translocation.

Gould & Cramer (1977), working with Escherichia coli, challenged this explanation of the role of valinomycin and SCN⁻ by demonstrating (in the absence of permeant ions) that at high cell/0₂ ratios, when the calculated membrane potential was energetically insignificant, the measured →H⁺/O⁺ ratio did not remotely attain its limiting stoichiometric value. Further, the stoichiometry of H⁺ ejection following the addition of a second oxygen pulse immediately after the first was unchanged. We have therefore re-investigated the role of so-called 'permeant' ions, as permeant agents, as part of the pathway of H⁺ transfer in membrane energy-coupling processes (Kell, 1979; Kell & Morris, 1981a; Kell et al., 1981).

P. denitrificans N.C.I.B. 8944 was grown and maintained as described previously (McCarthy et al., 1981). Mid-exponential-phase cultures were washed three times and resuspended at approx. 3 mg dry weight/ml in a 6 ml reaction mixture containing 150 mm KC1/0.25 mm glycollglycine, pH 6.5 plus 80 μg of carbonic anhydrase/ml. The potentiometric system was as described by Kell & Morris (1981b), and O₂ pulses were delivered as air-saturated KCl in the usual way (Scholes & Mitchell, 1970). The following results were obtained: (1) The number of measurable H⁺ ions translocated across the bacterial membrane, ∆H⁺, increased linearly with the size of the O₂ pulse from 4.7 to 47 ng-atom of O₂. (2) More than 90% of the observed H⁺ had been pumped across the bacterial membrane in that these protons were not observed when carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, 2 μM) was present. (3) This pattern was unchanged when the cell concentration was decreased by a factor of 3 or when NaCl or choline chloride were substituted for the KCl. (4) The →H⁺/O⁺ ratio obtained when 100 mm-KSCN was added increased from approx. 2 to approx. 7.5; the relative kinetics of the pH traces was similar to those obtained by Scholes & Mitchell (1970). (5) Low concentrations (10–60 μM) of sodium tetraphenylborate increased the observed →H⁺/O⁺ ratio approx. 2-fold. This could not have been caused by electrophoretic movement of tetraphenylborate from inner to outer bulk aqueous phases, since (a) the number of 'extra' H⁺ ions observed was greater than the number of free intracellular tetraphenylborate ions, and (b) the number of 'extra' H⁺ ions observed in the bulk phase at a given tetraphenylborate concentration was independent of the size of the oxygen pulse when this was varied between 14 and 42 ng-atoms. The appearance of these 'extra' protons was again sensitive to FCCP.

It may be concluded that, in the absence of compounds such as SCN⁻, protons translocated during electron transport pass directly to membrane-located sinks such as the ATPase (Hanselmann, 1974) via a non-bulk-phase pathway. The residual H⁺ ions observable in the bulk phase under these conditions are thus not used for membrane energy coupling. Proton ejection from the bulk phase external to the organisms may be stimulated both by blocking formation of the transmembrane field (electrophoretic mechanism) and by directly interfering with the function of membrane-located proteinaceous devices which normally act to channel pumped H⁺ ions to their membrane-located energetic sinks (non-electrophoretic mechanism). It is concluded from such measurements of respiration-driven H⁺ translocation that a non-osmotic mode of protonmotive energy coupling is exploited by P. denitrificans, in accordance with a previously outlined model (Kell, 1979; Kell & Morris 1981a; Kell et al., 1981).

We thank the Science and Engineering Research Council for generous financial support.


Characterization of succinate dehydrogenase from Micrococcus lysodeikticus

RICHARD CAMMACK,* BRIAN CROWET and PETER OWEN†
*Department of Plant Sciences, King's College, 68 Half Moon Lane, London SE24 9JF, U.K., and †Department of Microbiology, Moyne Institute, Trinity College, Dublin 2, Ireland

Succinate dehydrogenase is an integral part of the respiratory chain of Micrococcus lysodeikticus (Pollock et al., 1971). A specific antisera to this enzyme has been produced in rabbits after separating the enzyme by a crossed immunoelectrophoresis technique (B. Crowe & P. Owen unpublished work). The enzyme was then precipitated selectively from an extract of M. lysodeikticus membranes in 4% Triton X-100. The complex contained four polypeptides, of M, 72000, 30000, 17000 and 15000. By using cells grown on radioactively labelled riboflavin it was found that the largest subunit contains covalently bound flavin. The precipitated complex also contained a b-type cytochrome with maximum absorption (reduced—oxidized at 77K) at 556nm.

Vol. 10
As with other succinate intermediate of the Ca\(^{2+}\)-translocating ATPase that occurs in vesicles with that this Golgi-vesicle phosphoprotein is a phospho-enzyme of a membrane protein of apparent molecular weight 65,000. However, in comparison with the Ca\(^{2+}\)-dependent ATPase of the sarcoplasmic reticulum, which exemplifies the properties of well-characterized Ca\(^{2+}\)-translocating enzymes, the response of the Golgi-vesicle phosphoprotein to Ca\(^{2+}\) ions is atypical. Specifically, we have shown that removal of Ca\(^{2+}\) with EGTA led to a 3-fold increase, relative to controls without EGTA, in the amount of \(^{32}\)P incorporated into the 65,000 mol.wt. Golgi-vesicle protein after 20s incubation with \(\gamma\)-\(^{32}\)P-ATP. Conversely, the addition of 100\(\mu\)M-Ca\(^{2+}\) caused a halving of the \(^{32}\)P incorporation (West & Clegg, 1981).

The Ca\(^{2+}\)-dependence of the sarcoplasmic reticulum ATPase arises from the requirement for saturation of its Ca\(^{2+}\)-binding sites before it can react with ATP to form a phospho-enzyme intermediate; consequently, removal of Ca\(^{2+}\) by using EGTA prevents phospho-enzyme formation (Hasselbach, 1974; MacLennan & Holland, 1975). Clearly, if our hypothesis concerning the involvement of the 65,000 mol.wt. Golgi-vesicle phosphoprotein in Ca\(^{2+}\)-translocation is to retain credibility, we must further propose that the mechanism of ATP-dependent Ca\(^{2+}\) transport by the mammalian and plant Golgi membrane differs from that demonstrated in the sarcoplasmic-reticulum system. In

**Fig. 1. E.s.r. spectra, measured at 12 K, of succinate dehydrogenase--antibody precipitate from M. lysodeikticus membranes:**

(a) oxidized with 0.1 mm-K\(_{3}\)Fe(CN)\(_6\); (b) reduced with 20 mm-succinate for 5 min at 20°C (the signal at g = 1.93 is strongly saturated at this temperature); (c) reduced with 3 mm-Na\(_2\)S\(_2\)O\(_4\)+0.03 mm-Methyl Viologen (the signal at g = 2.0 in this spectrum is the reduced Methyl Viologen radical).

Conditions of measurement: microwave power 20 mW; frequency 9.18 GHz; modulation amplitude 0.1 mT.

---

**Ca\(^{2+}\)-dependent ATPase of rat mammary-gland Golgi vesicles: influence of Ca\(^{2+}\) and Mg\(^{2+}\) on the formation and breakdown of a phospho-enzyme intermediate**

DAVID W. WEST and ROGER A. CLEGG

Department of Biochemistry, Hannah Research Institute, Ayr KA6 5HL, Scotland, U.K.

We have reported that incubation of rat mammary-gland Golgi vesicles with \(\gamma\)-\(^{32}\)P-ATP leads to the transient phosphorylation of a membrane protein of apparent molecular weight 65,000 (West & Clegg, 1981). This phosphorylated protein has some properties in common with phospho-enzyme intermediates of a number of ATP-dependent enzymes (Yamamoto & Tonomura, 1968; Mackinose, 1969; Bastide et al., 1973; Katz & Blöstein, 1975). On the basis of these properties, we have hypothesized that this Golgi-vesicle phosphoprotein is a phospho-enzyme intermediate of the Ca\(^{2+}\)-translocating ATPase that occurs in the Golgi-vesicle membrane (West, 1981). However, in comparison with the Ca\(^{2+}\)-dependent ATPase of the sarcoplasmic reticulum, which exemplifies the properties of well-characterized Ca\(^{2+}\)-translocating enzymes, the response of the Golgi-vesicle phosphoprotein to Ca\(^{2+}\) ions is atypical. Specifically, we have shown that removal of Ca\(^{2+}\) with EGTA led to a 3-fold increase, relative to controls without EGTA, in the amount of \(^{32}\)P incorporated into the 65,000 mol.wt. Golgi-vesicle protein after 20s incubation with \(\gamma\)-\(^{32}\)P-ATP. Conversely, the addition of 100\(\mu\)M-Ca\(^{2+}\) caused a halving of the \(^{32}\)P incorporation (West & Clegg, 1981).

The Ca\(^{2+}\)-dependence of the sarcoplasmic-reticulum ATPase arises from the requirement for saturation of its Ca\(^{2+}\)-binding sites before it can react with ATP to form a phospho-enzyme intermediate; consequently, removal of Ca\(^{2+}\) by using EGTA prevents phospho-enzyme formation (Hasselbach, 1974; MacLennan & Holland, 1975). Clearly, if our hypothesis concerning the involvement of the 65,000 mol.wt. Golgi-vesicle phosphoprotein in Ca\(^{2+}\)-translocation is to retain credibility, we must further propose that the mechanism of ATP-dependent Ca\(^{2+}\) transport by the mammalian and plant Golgi membrane differs from that demonstrated in the sarcoplasmic-reticulum system. In