2P.24 Kinetics of oxidative phosphorylation catalyzed by inside-out plasma membrane vesicles of *Paracoccus denitrificans*

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Coupled inside-out plasma membrane vesicles of *Paracoccus denitrificans* are capable of proton-motive force (*pmf*)-required, *pmf*-generating ATP hydrolysis [1]. The steady-state ATP hydrolysis, catalyzed by *P. denitrificans* $F_O \cdot F_1$ ($PF_O \cdot F_1$) proceeds via compulsory sequential mechanism where ADP leaves the enzyme–products (ADP and Pi) complex first followed by irreversible release of Pi [2]. Inasmuch operation of $PF_O \cdot F_1$ ATPase (synthase) is macroscopically reversible it seems imperative to reconcile the steady-state kinetics of *pmf*-generating ATP hydrolysis with that of *pmf*-utilising ATP synthesis. To reach this goal the dependencies of the initial steady-state rates of oxidative phosphorylation on ADP and Pi concentrations and on *pmf* were measured. ATP synthesis rate showed simple hyperbolic dependence on either substrate (within the concentration ranges of 1.5–50 μM for ADP and 10–500 μM for Pi) with no mutual dependence of apparent K_m values thus suggesting random formation of the enzyme–ADP·Pi complex. When the 'third substrate', *pmf* was varied by limitation of succinate or NADH oxidation rates an apparent 'ping-pong' mechanism was evident: a decrease of the maximal rates caused by a decrease of *pmf* resulted in proportional decrease of apparent K_m values for either Pi or ADP. This pattern suggests that an irreversible step, presumably ATP formation at the enzyme active site, precedes the involvement of *pmf* in the overall reaction (likely at the product release step). Comparison of the steady-state kinetics of ATP hydrolysis [2] and synthesis shows that macroscopic reversibility of the $PF_O \cdot F_1$ ATPase (synthase) reaction cannot be ascribed to operation of single microscopically reversible enzyme species. We propose that apparent equilibrium between any given *pmf* and intracellular (intramitochondrial) phosphoryl potential ([ATP]/[ADP]·[Pi]) is maintained by 'futile cycle' of ATP synthesis and hydrolysis catalyzed by kinetically (and structurally) distinct $F_O \cdot F_1$ species.

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References

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2P.25 Structural basis for the ion selectivity of F-ATP-synthase c-ring rotors

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F_1F_O ATP-synthases are the most prominent ATP-producing enzymes across all life forms, from microbes to humans. They synthesize ATP in eukaryotic mitochondria and chloroplasts, as well as in the bacterial cytoplasm, by catalyzing the reaction between ADP and inorganic phosphate. To derive the energy required for catalysis, ATP synthases use the electrochemical gradient of protons or sodium ions across the membranes where they reside. Conversely, F_1F_O ATP synthases can hydrolyze ATP and thus serve as membrane ion pumps. Because ATP is the main energy source in living cells, this coupled process of synthesis/hydrolysis of ATP and Na^+/H^+ transport is of great importance, but remains poorly understood. In this work, we analyze the selectivity for H^+ or Na^+ of the membrane-embedded ion-binding subunit in the F_O domain, referred to as the c-ring rotor. We focus on the c_{11}-ring from *Ilyobacter tartaricus* and the c_{15}-ring from *Spirulina platensis*, and estimate their binding selectivity through DCCD labelling as a function of pH and salt concentration. While the c_{15}-rotor is shown to be highly proton selective, the c_{11}-ring is able to bind both Na^+ and H^+. Using molecular dynamics simulations and free energy calculations, we elucidate the structural and energetic basis for the distinct selectivity of these rotors, as well as of a series of mutants in which that selectivity is reversed towards H^+ or Na^+ binding.

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2P.26 Single, double and triple alanine to glycine replacements in the AxAxAxA motif of alkaliphilic *Bacillus pseudofirmus* OF4 *c*-subunits affect *c*-ring stability, change both monomer and *c*-ring mobility in SDS-PAGE and lead to deficits in ATP synthesis

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The F_1F_O-ATP synthases/ATPases that are used to support ATP synthesis during non-fermentative growth of some extremely alkaliphilic *Bacillus* species have special features that distinguish them from ATP synthases of neutralophiles or sequestered organelles, non-respiratory alkaliphiles or less extreme alkaliphiles [1]. One of the major deviations observed in the *c*-subunit, which forms the oligomeric *c*-ring rotor of the synthase, is the replacement of a GxGxGxG motif that is near the center of the N-terminal helix of most other c-subunits [2] with an AxAxAxA motif in alkaliphilic *Bacillus pseudofirmus* OF4. Replacement of the alkaliphile version with a consensus motif, GxGxGxG, resulted in severe deficits in malate growth and ATP synthase activity at both pH 7.5 and 10.5, without a comparable deficit in detergent-stimulated ATPase activity (i.e. total ATPase activity). Single mutants and sample double and triple mutants were shown to have more modest defects in malate growth than the quadruple mutants [2]. Here, we took advantage of the stability of the wild-type *B. pseudofirmus* OF4 *c*-ring to investigate an enlarged panel of ^{16}AxAxAxA^{22} mutants of *B. pseudofirmus* OF4 to assess changes in content and mobility of the rings on SDS-PAGE gels. ATP synthases from 4 single, 4 double, 1 triple and the 1 quadruple mutant were his-tagged on the β-subunit, purified and fractionated on SDS-PAGE gels with no treatment, treatment with trichloroacetic acid (TCA) to monomerize the ring, or after extraction with lauryl sarcosine to purify the *c*-ring away from most of the other synthase subunits. A striking finding was the doublet *c*-ring band observed in the single A16G mutant on the gel; the less intense band was the approximate size of the wild-type ring and the more intense bottom band was below the wild-type. All mutants containing A16G mutations along with other mutations exhibited only the lower band. In addition, *c*-subunit monomers from mutants with A16G