

springs in Armenia), which was grown anaerobically at pH 7.0 under light, from succinate using different amino acids (glutamate, alanine, tyrosine, and glycine) as nitrogen sources have been studied. The maximal cell growth rate and H₂ production by *R. sphaeroides* were obtained when glutamate was used as nitrogen source. The H₂ production rate, in the presence of alanine and tyrosine, in comparison with glutamate, decreased about 3-fold. In the presence of glycine the bacterial growth, but not H₂ production was observed. This process was suppressed at the presence of the *N,N'*-dicyclohexylcarbodiimide (DCCD), the F₀F₁-ATPase inhibitor. The addition of DCCD (0.5 mM) in succinate–glutamate, succinate–tyrosine or succinate–alanine medium caused a decrease of H₂ production rates (about 50%).

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14P.7 EPR-HYSCORE study of quinone binding in respiratory nitrate reductase: Molecular basis for the adaptation to anaerobiosis–aerobiosis transition

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Nitrate reductase A (NarGHI) is a respiratory complex that is preferentially expressed under anaerobic conditions and in the presence of nitrate in *Escherichia coli* cells. This membrane-bound enzyme is then associated with formate dehydrogenase (FdnGHI) to constitute a redox loop enabling the energetic coupling between transmembrane electron and proton transfers. By shuttling electrons between these two complexes, quinones are key elements of this bioenergetic chain. NarGHI is physiologically able to oxidize either menaquinols or ubiquinols associated to anaerobic or aerobic growing conditions, respectively. However, due to the absence of quinone in the crystal structure of NarGHI, the number and location of the quinol binding sites were largely debated. By combining EPR spectroscopy and site-directed mutagenesis, we have recently shown that a semiquinone radical species can be stabilized in close vicinity of the distal heme *b_D* located in the NarI membrane subunit. This radical was identified as a menasemiquinone (MSQ) intermediate. Surprisingly, it exhibits the highest stabilization constant reported so far in respiratory enzymes [1,2]. To understand the molecular basis of this unusual stabilization, a multifrequency HYSCORE study was directly undertaken on NarGHI-enriched inner membrane vesicles (IMVs) [3]. Analysis of the ¹⁴N and ¹⁵N hyperfine couplings reveals that MSQ is specifically H-bound to a nitrogen atom which was assigned to the N_d imidazole nitrogen of the heme *b_D* axial ligand His66. Moreover, the EPR study of NarGHI-enriched IMVs purified from a menaquinone-deficient *E. coli* strain shows that endogenous ubisemiquinones (USQ) can also be detected. The use of ¹⁴N HYSCORE enabled to distinguish the USQ radicals bound to various membrane-bound enzymes, and to clearly identify the USQ species

bound to NarGHI. Noticeably, MSQ and USQ bind in a single site of the NarGHI complex in a similar mode involving one of the His heme *b_D* ligand [4]. This work provides the first spectroscopic evidence to address at the molecular level the question of the adaptation of an anaerobic enzyme to oxygenic conditions.

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14P.8 Novel electrochemical methods to characterise ubiquinol oxidase activity in native-like model-membrane systems

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Bacteria have highly diverse and highly branched respiratory chains, which consist of a range of enzymes that transfer electrons from many different substrates into a common pool of lipid soluble electron carriers, known collectively as quinones. In the aerobic respiratory pathway of *Escherichia coli* there are two types of ubiquinol oxidases that catalyse the oxidation of ubiquinol to ubiquinone and reduce molecular oxygen to water. The ubiquinol oxidase cytochrome *bo₃* (*cbo₃*) is structurally related to the mammalian terminal oxidase, cytochrome *c* oxidase, and its catalytic cycle is coupled to the pumping of protons across the membrane. We have developed two native-like membrane systems to investigate different bioenergetic aspects of *cbo₃* using electrochemical methods. In the first model system, planar orientated membranes are formed onto gold electrodes functionalised with cholesterol derivatives. *Cbo₃* activity in these planar membranes is monitored using cyclic voltammetry with electron transfer to *cbo₃* mediated by the ubiquinol/ubiquinone (UQ) pool. Using impedance spectroscopy, the diffusion rate of UQ is found to be orders of magnitude slower than accepted values for lateral diffusion. It is therefore hypothesised that these rates represent perpendicular diffusion of UQ 'head-group' across the membrane, corresponding to a 'flip' time between 0.05 and 1 s. The apparent *K_M* of *cbo₃* for oxygen was measured at $1.1 \pm 0.4 \mu\text{M}$, in good agreement with literature values for whole cell experiments and for purified *cbo₃*. Increasing the concentration of lipophilic UQ above 5–10 pmol/cm² in the membrane, either by incorporating UQ in the membranes before assembly of the planar membrane or adding it *in situ* during the voltammetric experiments, leads to a decrease in *cbo₃* activity. Analysis of the data indicates that *cbo₃* is inhibited by ubiquinol at high concentrations (substrate inhibition), but not by ubiquinone (product). In the second model-membrane system, vesicles are adsorbed intact on the electrode surface. By incorporating a pH-sensitive fluorescent dye inside the vesicles, the generation of a proton gradient (DpH) by *cbo₃* is monitored. The rate of pH increase inside the vesicle is measured after *cbo₃* is electrochemically activated. After correcting for proton leakage into the vesicles, the proton pumping activity (in DpH/s) of *cbo₃* is shown to be linearly related to DpH.

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