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14P.15 Supramolecular organization of the aerobic respiratory chain of *Escherichia coli*

Pedro M.F. Sousa[1,2], Sara T.N. Silva[1], João N. Carita[2], Filipe A.S. Santos[1], Ana M.P. Melo[1]
[1]*ECO-BIO, Instituto de Investigação Científica Tropical, Av. da República, 2784-505 Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
E-mail: pedrosousa@itqb.unl.pt

The composition of the *Escherichia coli* aerobic respiratory chain varies according to the oxygen tension of growth and relies essentially on the expression of at least five proteins, namely type I and II NADH:quinone oxidoreductases, succinate:quinone oxidoreductase, cytochrome *bd* oxygen reductase and cytochrome bo_3 oxygen reductase. The arrangement of these enzymes in the cytosolic membrane is still controversial, mostly due to increasing evidence suggesting their organization into supramolecular assemblies, in respiratory chain complexes from other organisms. To investigate if such organization could be detected in the aerobic respiratory chain of this bacterium, membranes of *E. coli* K-12 solubilized with digitonin were analyzed by BN-PAGE followed by *in-gel* activity and heme staining detection. The resulting bands were also loaded into 2D-Tricine-SDS-PAGE and 2D-CN-PAGE, transferred to PVDF membranes and immunodecorated with polyclonal antibodies against type I NADH:quinone oxidoreductase, succinate:quinone oxidoreductase and cytochrome bo_3 oxygen reductase. The expected trimeric and dimeric assemblies of succinate:quinone oxidoreductase and cytochrome bo_3 were detected as well as four bands presenting NADH:NBT oxidoreductase activity. Two of the NADH:NBT oxidoreductase stained bands stained also with antibodies against the type I NADH:quinone oxidoreductase, the succinate:quinone oxidoreductase and the cytochrome bo_3 oxygen reductase, strongly suggesting that the *E. coli* respiratory chain harbours at least a supercomplex containing these three enzymes. The BN-PAGE results are further corroborated by sucrose gradient analysis and gel filtration column purification [1].

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14P.16 Redox processes, energy transfer and molecular hydrogen production studies with *Escherichia coli* at different pH

Armen Trchounian
Yerevan State University, Department of Biophysics, Armenia
E-mail: Trchounian@ysu.am

Escherichia coli perform sugar or glycerol fermentation upon which redox processes, energy transfer and H_2 production are of interest. Two aspects are under our study: (1) In neutral and slightly alkaline medium *E. coli* carry out H^+–K^+-exchange through the F_0F_1-ATPase and the TrkA system when energy for K^+ uptake is transferred from F_OF_1 by dithiol-disulfide interchange between these complexes; data obtained support this idea. A relationship between H^+–K^+-exchange and H_2 production by formate hydrogen lyase (FHL) might be mediated by redox equivalent supply from formate for a dithiol–disulfide interchange. Moreover, proton-motive force generated by F_0F_1 may be required for transport of formate into cells and for FHL activity. However, in acidic medium, F_0F_1 is likely to be also necessary for H_2 production. (2) Two forms of FHL-1 and FHL-2, which constituted by formate dehydrogenase H encoded by the *fdh* operon and hydrogenases 3 (Hyd-3) or 4 (Hyd-4), encoded by the *hyc* and *hyf* operons, respectively, are responsible for H_2 production. Since Hyd-3 and Hyd-4 are encoded by genes of different operons and are characterized by different subunit composition and organization in the membrane it is assumed that these forms are functionally active under different conditions and therefore they play distinct roles in bacteria. Our study with *E. coli* grown under glucose fermentation at neutral and slightly alkaline pH has shown that H_2 production was not observed in *fdhF* and *hyf*-mutants lacking Fdh-H large subunit or Hyd-4 subunits, respectively. Interestingly, production of H_2 was detected in various *hyc* mutants lacking Hyd-3 subunits, but it was not formed in the *hycB* mutant. These results suggest that H_2 production by *E. coli* involves FHL-2; HycB requirement subunit suggests that it represents a constituent of Fdh-H. The physiological role of FHL-2 may be required for generation of CO_2 to use in the formation of oxaloacetate from phosphoenolpyruvate during fermentation. At acidic pH H^+–K^+-exchange by *E. coli* was distinguished: K^+ uptake by the Kup system was markedly lower in *hyfR* and *hyfB-R* but not in *hycE* or *hyf A-B* mutants and H_2 production was significantly suppressed in the *hyc* but not *hyf* mutant. The *hyfB-R* genes are suggested to be expressed under low pH. These genes or their gene products are possible to interact with the gene coding for Kup or directly with Kup.

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14P.17 The type II NADH: Quinone oxidoreductase of *Mycobacterium tuberculosis*: A novel drug target for an age-old problem

Ashley J. Warman[1], Teresa Rito[1], Nicholas Fisher[1], Neil G. Berry[2], Paul M. O'Neill[2], Stephen A. Ward[1], Giancarlo A. Biagini[1]
[1]*Molecular and Biochemical Parasitology, Liverpool School of Tropical Medicine, University of Liverpool, L3 5QA, UK*
[2]*Department of Chemistry, University of Liverpool, L69 7ZD, UK*
E-mail: ajwarman@liverpool.ac.uk

The *Mycobacterium tuberculosis* (Mtb) respiratory chain presents unique antitubercular drug development opportunities. In addition to a 14 subunit Complex I, the Mtb Electron Transfer Chain contains a single subunit type II NADH:quinone oxidoreductase (Ndh). Transcriptional studies, animal infection models and biochemical analyses have shown this metabolic choke point to be essential [1,2]. It is a major contributor to the viability-sustaining membrane potential ($\Delta\Psi m$) regardless of metabolic state and consequently inhibition induces death in actively-growing and dormant Mtb. Targeting Ndh will mitigate failings of current therapies, circumventing current resistance mechanisms and sterilising dormant populations. Heterologously expressed Mtb Ndh has been successfully isolated from the double NADH-dehydrogenase knockout *E. coli* strain ANN0222 (*nuoB*::*nptl-sacRB*, *ndh*::*tet*, supplied by Prof. T. Friedrich, Freiburg) and is catalytically active. This activity is rotenone and piercidin A insensitive, however, in line with recent literature [1,2] Mtb Ndh is sensitive to the phenothiazines trifluoperazine and thioridazine. To probe the Ndh quinone binding site a conserved tryptophan (W276) postulated to be critical to enzyme–substrate interaction was mutated to Ala, Leu, and Phe. W276A and W276L mutants are inactive whilst W276F displays minimal activity suggesting that *pi-pi* stacking interactions between W276and substrate aromatic rings are critical to catalysis. A direct positive correlation between enzyme inhibition and Mtb bactericidal activity has been

established for a range of phenothiazine derivatives. Subsequently a miniaturised assay with robust performance measures has been developed for high throughput screening of small molecule inhibitors [3]. A focused screen of 11,000 compounds from a collection of 750,000 (Biofocus-DPI) has been undertaken and from these about 0.3% hits (active at <25 μM) have been identified. Dose response curves of hits show activity (IC_{50}) against Ndh at nM levels with corresponding sterilisation activity under both aerobic and anaerobic conditions. A number of structurally diverse templates have been prioritised and will be pursued using traditional medicinal chemistry QSAR.

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14P.18 The distal pterin of *Escherichia coli* nitrate reductase A (NarGHI) participates in a charge transfer relay that modulates enzyme activity

Gregory J. Workun[1], Matthew Solomonson[1], Thomas Spreter[2], Maryam Zarapour[1], Nasim Bouromand[1], Natalie C.J. Strynadka[2], Joel H. Weiner[1]
[1]*Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7*
[2]*Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3*
E-mail: joel.weiner@ualberta.cajoel.weiner@ualberta.ca

The current view of molybdoenzyme function is that the Mo atom plays the primary role in catalysis. We will present evidence that the pterin ring system can also play critical redox roles distinct from the redox reactions of the Mo. The bis-pterin molybdenum cofactor of *Escherichia coli* nitrate reductase A (NarGHI) contains tricyclic and bicyclic pterins that are proximal and distal to the FS0 [4Fe–4S] cluster, respectively. Site-directed mutagenesis, EPR spectroscopy, redox potentiometry, and protein crystallography were used to examine the assembly of a novel bicyclic distal pterin into the catalytic subunit (NarG). Inspection of available NarG protein structures reveals that the open pyran ring of the distal pterin is stabilized by hydrogen bonds between its hydroxyl oxygen and two conserved residues, NarG-S719 and NarG-H1163. The latter residue is also paired with a second conserved residue, NarG-H1184, forming a charge transfer relay between the pyran hydroxyl and three structurally-conserved water molecules. We have demonstrated that in a double mutant enzyme, NarG-S719A-H1163A, the pyran ring of the distal pterin is closed, rendering it similar to those of other bis-pterin molybdoenzymes of known structure. The NarG-S719A mutation has a less deleterious effect on enzyme function and molybdenum redox chemistry than either the NarG-H1163A mutant or the double mutant, indicating that the charge transfer relay plays a critical role in enzyme function. EPR spectroscopy in combination with potentiometric titrations indicates that NarG-H1163 and the charge transfer relay in which it participates play a critical role in defining redox chemistry and catalysis at the Mo atom. These results demonstrate the importance of residues contacting the organic component of the bis-pterin cofactor in controlling catalysis.

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