S1.L4

Arrayed lipid bilayer chambers for single-molecule transporter analysis

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Nano/micron-sized reaction chamber arrays (femtoliter-chamber arrays) enable highly sensitive and quantitative biological assays, such as single-molecule enzymatic assays [1, 2], digital PCR [3, 4], and digital ELISA [5]. However, the versatility of femtoliter-chamber arrays has been limited to reactions in aqueous solutions. In this presentation, I will introduce an arrayed lipid bilayer chamber system (ALBiC) that displays a sub-million femtoliter chambers, each sealed with a stable 4-μm diameter lipid bilayer membrane with extremely high efficiency (yield: ~99%). When reconstituted with a limiting amount of the membrane transporter proteins α-hemolysin or FoF1-ATP synthase, the chambers of the ALBiC exhibited stochastic and quantized transporting activities, demonstrating that the single molecule analysis of passive and active membrane transports is achievable with the ALBiC system. Thus, this new platform has vastly extended the versatility of femtoliter chamber arrays and could contribute to the understanding of the working mechanism of membrane proteins as well as to further analytical and pharmacological applications. If time allows, I would like to talk about new versions of the ALBiC that we recently developed. References 1. Rondelez Y, et al. (2005) Highly coupled ATP synthesis by F1-ATPase single molecules. Nature 433(7027):773–777. 2. Rondelez Y, et al. (2005) Microfabricated arrays of femtoliter chambers allow single molecule enzymology. Nature biotechnology 23(3):361–365. 3. Ottesen EA, Hong JW, Quake SR, & Leadbetter JR (2006) Microfluidic digital PCR enables multigene analysis of individual environmental bacteria. Science 314(5804):1464–1467. 4. Pekin D, et al. (2011) Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. Lab on a chip 11(13):2156–2166. 5. Kim SH, et al. (2012) Large-scale femtoliter droplet array for digital counting of single biomolecules. Lab on a chip 12(23):4986–4991.

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S1.O1

Channel formation by yeast F-ATP synthase and the role of dimerization in the mitochondrial permeability transition

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Purified F-ATP synthase dimers of yeast mitochondria display Ca^{2+}-dependent channel activity with properties resembling those of the permeability transition pore (PTP) of mammals [1]. After treatment with the Ca^{2+} ionophore ETH129, which allows electrophoretic Ca^{2+} uptake, isolated yeast mitochondria undergo inner membrane permeabilization due to PTP opening [2]. Yeast mutant strains ΔTIM11 and ΔATP20 (lacking the e and g F-ATP synthase subunits, respectively, which are necessary for dimer formation [3]) display a striking resistance to PTP opening. These results show that the yeast PTP originates from F-ATP synthase, and indicate that dimerization is required for pore formation in situ.

References

[1] V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, et al., Dimers of mitochondrial ATP synthase form the permeability transition pore., Proceedings of the National Academy of Sciences of the United States of America. 110 (2013) 5887–92. http://dx.doi.org/10.1073/pnas.1217823110.
[2] A. Yamada, T. Yamamoto, Y. Yoshimura, S. Gouda, S. Kawashima, N. Yamazaki, et al., Ca2+-induced permeability transition can be observed even in yeast mitochondria under optimized experimental conditions., Biochimica et Biophysica Acta. 1787 (2009) 1486–91. http://dx.doi.org/10.1016/j.bbabio.2009.07.001.
[3] I. Arnold, K. Pfeiffer, W. Neupert, R. a Stuart, H. Schägger, Yeast mitochondrial F1F0-ATP synthase exists as a dimer: identification of three dimer-specific subunits., The EMBO Journal. 17 (1998) 7170–8. http://dx.doi.org/10.1093/emboj/17.24.7170.

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S1.O2

Mechanism of the F_o-stepping motor revealed by single-molecule experiments

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Single-molecule experiments of the *Escherichia coli* F_oF_1 ATP synthase reveal for the first time the existence of an F_o-dependent power stroke that can rotate the c-ring up to a maximum of ~36°, the equivalent of one c-subunit, in the ATP synthase direction against the force of F_1 ATPase-driven rotation. Evidence supports a grab-and-push mechanism in which subunit-a grabs one subunit-c near the membrane–cytoplasm interface, then pushes the c-ring as the result of a protonation-dependent conformational change of subunit-a. The location at which subunit-a grabs was identified by mutations that eliminated charged residues. These mutations decreased the ability of subunit-a to grab the c-ring, and adversely affected ATP synthesis and proton translocation in the ATP synthesis direction indicating the participation of these residues in a gating mechanism for ATP synthesis-dependent proton translocation.

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S1.P1

Modulation of F-ATP synthase by pH: Role of His112 protonation of OSCP

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The mitochondrial FOF1ATP synthase forms long rows of dimers in the inner membrane cristae and is composed of the catalytic F1 and the membranous FO sectors linked by central and peripheral

stalks. Recently it was demonstrated (i) that cyclophilin D binds to the FO OSCP subunit, resulting in partial enzyme inhibition; (ii) that CyPD binding requires high Pi, while the CyPD inhibitor CsA displaces CyPD from OSCP resulting in enzyme reactivation; and (iii) that ATP synthase dimers generate Ca^{2+}-dependent currents indistinguishable from those of the permeability transition pore (PTP), suggesting that the PTP forms from a Ca^{2+}-dependent conformational change of FOF1 dimers [1]. These findings imply that many modulators of the PTP may act on the ATP synthase. The most potent PTP inhibitors are H^+; indeed, the pore is blocked at acidic matrix pH that also promotes CyPD release from the inner membrane. Diethylpyrocarbonate (DPC) prevents PTP inhibition by H^+ through carbethoxylation of His residues, and also prevents the release of CyPD from the inner membrane induced by acidic pH [2]. We found that DPC also prevents the release of CyPD from ATP synthase induced by acidic pH. As is the case for the PTP, the effect of DPC was reversed by hydroxylamine, indicating that it can be traced to carbethoxylation of His residue(s). This in turn suggests that reversible protonation of the unique histidyl residue of OSCP (bovine His112) may play a critical role in modulation of the CyPD–ATP synthase interaction. Consistently, when OSCP subunit separated from mitochondria treated with DPC was digested with trypsin and analysed by ESI-MS, a mass shift of +72 Da of the OSCP 95-113 peptide was determined, which is consistent with carbethoxylation of the unique His112. In conclusion, DPC is proving very useful to address the role of OSCP His112 in modulation of CyPD binding to ATP synthase/PTP by matrix pH, which will be further addressed by mutagenesis.

References

[1] V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G.D. Glick, V. Petronilli, M. Zoratti, I. Szabó, G. Lippe, P. Bernardi, Dimers of mitochondrial ATP synthase form the permeability transition pore, PNAS, 110 (2013) 5887–92.
[2] A. Nicolli, E. Basso, V. Petronilli, R.M. Wenger, P. Bernardi, Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel, J. Biol. Chem., 271 (1996) 2185–92.

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S1.P2

The mechanism of binding of an intrinsically disordered mitochondrial inhibitor protein to F1-ATPase

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IF1 is an 84 amino acid length peptide that inhibits the ATP hydrolysis activity of F1Fo-ATPase. In solution, IF1 forms homodimers with two domains: a coiled-coil dimerization domain comprising residues 48–84, and a N-terminal inhibitory domain, from residues 1–45, that is intrinsically disordered. In the structure of bovine F1-ATPase inhibited with residues 1–60 of the bovine inhibitor protein IF1, one inhibitor protein (I1-60) interacts with five of the nine subunits of F1-ATPase, and I1-60 is bound tightly at the αDPβDP catalytic interface. Formation of the inhibited complex requires ATP hydrolysis. It has been proposed that the first interaction between the inhibitor and F1-ATPase is with the βE-subunit (the most open state), and that the inhibitor becomes entrapped progressively as two ATP molecules are hydrolysed. We have solved three novel F1–IF1 structures that support this proposal. Crystals of F1-ATPase were grown in the presence of a large molar excess of one of three inhibitors: I1-60His, I1-60His K39A, and I1-60His F22W Y33W. The resultant complexes all have multiple copies of the inhibitor bound to one F1-ATPase molecule. The structures reveal the binding cycle of the inhibitor to F1-ATPase showing how the intrinsically disordered inhibitory domain of IF1 becomes gradually more ordered as it interacts with F1-ATPase. The folding pathway of IF1 is shown with a greater level of structure possible for IF1 as its interactions with F1-ATPase become progressively more extensive.

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S1.P3

Regulatory conformational changes of the epsilon subunit in single FRET-labeled F_1 and F_oF_1-ATP synthase

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Subunit ε is an intrinsic regulator of the bacterial F_oF_1-ATP synthase. The C-terminal domain of ε can extend into the central cavity formed by the a and b subunits as revealed by the recent X-ray structure of the F_1 portion of the *Escherichia coli* enzyme [1]. This insertion blocks the rotation of the central g subunit and, thereby, prevents wasteful ATP hydrolysis. We developed an experimental system including a microfluidic single-molecule trap [2] to observe how epsilon inhibits the F_1 portion and the holoenzyme F_oF_1-ATP synthase. Labeling the C-terminal domain of the ε and g subunits specifically with two different fluorophores for single-molecule Förster resonance energy transfer (smFRET) allowed monitoring of the conformation of ε of the F_1 portion [3] or the reconstituted enzyme in real time [4].

References

[1] G. Cingolani, T. M. Duncan, Structure of the ATP synthase catalytic complex (F1) from *Escherichia coli* in an autoinhibited conformation, Nat. Struct. Mol. Biol. 18 (2011) 701–707.
[2] S. D. Bockenhauer, T. M. Duncan, W. E. Moerner, M. Börsch, The regulatory switch of F_1-ATPase studied by single-molecule FRET in the ABEL Trap, Proc. SPIE 8950 (2014) 89500H.
[3] M. Börsch, T. M. Duncan, Spotlighting motors and controls of single F_oF_1-ATP synthase, Biochem. Soc. Trans. 41 (2013) 1219–1226.
[4] T. M. Duncan, M. G. Düser, T. Heitkamp, D. G. G. McMillan, M. Börsch, Regulatory conformational changes of the e subunit in single FRET-labeled F_oF_1-ATP synthase, Proc. SPIE 8948 (2014) 89381J.

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S1.P4

Redox regulation of cyanobacterial chimera F1-ATPase comprises interplay between the γ-subunit "neck" region and the turn region of the βDELSEED-loop

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ATP synthase (FOF1) is an important enzyme for energy conversion in the cell and can be found in energy-transducing membranes of bacteria, mitochondria and chloroplasts. The enzyme