Application of fluorescent protein ATeam for ATP level measurements in living bacteria
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ATeam is a fluorescent ATP indicator based on FRET and composed of the ε subunit of bacterial F_{0}F_{1}-ATP-synthase sandwiched by the cyan and yellow fluorescent proteins [1]. It turned to be convenient for visualizing of ATP levels inside living eukaryotic cells, but to our knowledge it was never used in experiments on bacteria. The intracellular ATP level is a key parameter in bacterial bioenergetics, so we consider ATeam as an efficient tool in this field.

Using ATeam probe, we developed a new method for ATP level measurement in bacterial living cells. We monitored the probe response in wild type Escherichia coli cells expressing ATeam, and tested its adequacy with standard chemiluminescence method. In this system, as expected, the signal rate increased after glucose addition and was reduced after addition of ATP synthase inhibitor potassium arsenate. The addition of respiratory substrate succinate also led to signal rate increase, and the following addition of protonophore resulted in decrease of signal rate. When uncoupling preceded the succinate addition, the signal rate increase was not observed.

With new approach, we also studied dynamics of ATP levels in Escherichia coli Δunc mutant with deleted ATP synthase operon. Glucose and arsenate additions influenced the signal rate the same way as in wild type. Addition of succinate to mutant cells unexpectedly led to increase of signal rate, which, however, was half less than that after glucose addition.

In general, our data correspond well to general knowledge of bacterial energetic metabolism, so our method seems suitable for bacterial bioenergetic studies.

Reference

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1P25

Predicted topology of the ion-conducting subunit-a of the membrane motor of the ATP synthase
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Subunit-a plays a critical role in the rotary mechanism of the ATP synthase F_{0} membrane motors. First, it functions as the stator against which the c-subunit ring rotates, thus producing the mechanical torque required for catalysis in the soluble F_{1} motor. Second, it provides access pathways for protons or Na⁺ ions to and from the c-ring binding sites, thereby coupling the rotation of the c-ring to the transmembrane electrochemical gradient. The structure of subunit-a is to date unknown, but it is generally accepted that a bundle of four transmembrane helices, TM2-TM5, lie in proximity to the c-ring. However, the topology of this bundle, which likely influences the ion-translocation mechanism, remains unclear. Here, we first use the structure-prediction algorithm Rosetta to construct a topological model of the E.coli aTM2-aTM5 helix bundle that is optimally consistent with existing cysteine cross-linking data for the isolated subunit-a. We also construct a model of the a/c-interface, guided by novel cross-linking data between genetically fused c2-subunits and subunit-a, plus previously reported cross-linking and accessibility data involving aTM4-aTM5 and the c-subunit. Our results demonstrate that the aTM2-aTM5 transmembrane bundle is organized in a clockwise manner viewed from the cytoplasm. In our a/c-complex model we identify two half-channels from the center of the membrane, one at the a/c-interface extending towards the cytoplasm and another within the subunit-a bundle, open to the periplasmic environment.

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ATP synthase from rat liver plasma membrane
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Over the past few years, a growing number of reports, including our work [1], have described the presence of F_{0}F_{1}-ATP synthase subunits at the surface of a wide variety of cells, including hepatocytes. Expression of ATP synthase at the cell surface is related to different biological effects; in liver it mediates HDL endocytosis through the activation of the G protein-coupled purinergic receptor P2Y13 [2].

A question regarding the ectopic ATP synthase is its sub-localization at the plasma membrane. Hepatocytes are polarized due to the presence of the basolateral and canalicular membranes, devoted for uptake from the portal blood and bile secretion, respectively. Here we have compared two preparations of plasma membrane [1, 3] from rat liver, which have been characterized for their content of basolateral membrane markers. Both preparations were fractionated using different concentrations of digitonin, followed by hr CN PAGE, and the presence of F_{0}F_{1} complexes was established by in-gel activity staining and immunoblotting.

The results indicate that the plasma membrane preparation enriched in basolateral proteins contains higher amounts of complete and active F_{0}F_{1} complexes, in accordance with their specific function to modulate HDL uptake on hepatocyte surface. Moreover, we have demonstrated that, independently of the extraction protocol, ATP synthase complexes display a similar molecular weight to the monomeric form of the mitochondrial ATP synthase. This confirms that, in the plasma membrane, the F_{0}F_{1} complexes do not associate into dimers and oligomers as they do in mitochondria.

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References
F-type ATP synthases typically harbor c-subunits of approximately 8-9 kDa that form oligomeric rings of 8-15 monomers with one ion-binding site per subunit. Each c-subunit in the ring forms a hairpin-like structure in the membrane with an inner α-helix, a cytoplasmatic loop and an outer α-helix. The membrane rings in eukaryotic V-type ATPases and archaeal A-type ATP synthases often have analogous but duplicated c-subunits with four instead of two transmembrane α-helices, harboring either one or two ion-binding sites per subunit. The anaerobic, acetogenic bacterium *Acetobacterium woodii* has a Na⁺-driven F-type ATP synthase, which contains a unique rotor composed of c-subunits of both types and transmembrane topologies. The enzyme is encoded by an atp-operon comprising 11 genes (*atpBEFEGHAGDC*) with three genes coding for two different c-subunits: atpE codes for a duplicated c-subunit, c1, whereas atpD and atpE code for single hairpin c-subunits, c2 and c3. We solved the structure of the A. woodii c-ring by X-ray crystallography at 2.4 Å resolution. This high resolution structure confirms earlier indications that this c-ring consists of c1:c2:c3 subunits in a 1:9 stoichiometry. All c2:c3-subunits (single hairpin) exhibit a Na⁺-binding site. A conserved glutamate residue in the locked conformation, halfway along the outer α-helix, three other carbonyl/hydroxyl groups and a water molecule are involved in Na⁺-coordination. Interestingly, the c1-subunit (double hairpin) contains only one Na⁺-binding site, of identical make-up. In the second site, a glutamine replaces the conserved glutamate, and prevents Na⁺-binding. Hence, the c-ring from the A. woodii ATP synthase carries 10 sodium ions across the membrane per 360-degree rotation. Similar to some of the V-type c-subunits, the c1-subunit has an N-terminal extension, which is also visible by atomic force microscopy. In summary, we present the first atomic-resolution structure of a heteromeric ATP synthase ring with intriguing mechanistic implications.

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**1P27**

**Evaluation of neuronal cell energetic status using theoretical description of FoF1-ATPsynthase catalytic cycle**  
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Energetic status of the cell is one the most important factors of its normal physiological functioning. Meanwhile its experimental evaluation is not always possible and reasonable due to high costs and invasiveness. Theoretical description of ATP production in the cell can be possible using existing data about the structure, functioning and spatial distribution of energoproducing systems. The FoF1-ATPsynthase is one of the key enzymes in energy supplying in almost all living systems. This enzyme is an attractive research target due to its high mechanical complexity. Nevertheless, some key issues regarding energy conversation during the FoF1-ATPsynthase catalytic cycle are still unclear.

The issue of rotational catalysis convertibility arises in the step that describes the rotor movement and proton translocation. This is one of the features that causes difficulty in the creation of a universal model of ATP synthesis. In this work, we provide a theoretical description of FoF1-ATPsynthase catalytic cycle using combined mathematical approaches. Thus, the mechanism of Fo can be nominally described as the complexity of the rotation of proton subunits and proton translocation through membrane half-channels, which is an obligatory step in the transformation of the accumulated energy. The description of the above processes can yield the time of proton translocation and the mechanism of energy transformation and its transition to ATP-binding sites. Langevin dynamics is used for the rotation of the central protein core and the Monte-Carlo method helps to model nucleotide and proton binding. This approach is the first in which both ATP synthesis and hydrolysis can occur depending on the nucleotide concentration and system conditions. The calculated rates are close to the experimentally measured rates of ATP functioning. The model has been formalized as a computer simulation Program of ATP Synthesis and Hydrolysis (PASH) that allows researchers to evaluate ATP production both for one enzyme and for different types of cells. Flexibility of parameter settings in synthesis and hydrolysis of active enzymes and their regulation allows universal application of this approach in the evaluation of cell energetic status.

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**1P28**

**Structure of the F₀/V₀-hybrid ATP synthase rotor ring from Acetobacterium woodii at 2.4 Å resolution**  
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ADP-inhibition is well known phenomenon common to all F₁-ATPases so far examined. The entrapment of ADP-Mg at catalytic sites stops catalytic turnover. The inhibition by the ε subunit is also known as a common mechanism among F₁-ATPases from bacteria and plants. The ε subunit inhibits ATPase activity of F₁-ATPase by changing its conformation. Both of the inhibitions may concern with the regulation of ATP synthase. It has not been settled if inhibition by the ε is due to the stabilization of ADP-inhibition [1, 2] or not [3]. Since both inhibit ATPase activity, it is difficult to distinguish which inhibition is responsible for the inhibition at the particular moment.

We have constructed overexpression systems of αβγε complex and ε subunit of F₁-ATPase from *Bacillus subtilis* (BF₁), and performed kinetic characterization of αβγε complex (BF₁(-ε)) and reconstituted αβγεεε complex (BF₁(+ε)). The ADP-inhibition of BF₁ was so strong that it was activated more than ×100-fold by the addition...