valinomycin or Complex I inhibitor, rotenone. Under such conditions mitochondrial reticulum formed short bulky spheres, nucleoids clustered inside however maintained their autonomy.

This work was supported by the Czech Scientific Foundation GACR grant no. P304/10/P204 (to A.D.), P302/10/0346 (to P.J.), and the Czech Ministry of Education ME09029 (to P.J.).

References

doi:10.1016/j.bbabio.2012.06.405

21P2
Bacterial oxygen production in the dark
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Nitric oxide (NO) and nitrous oxide (N₂O) are among nature’s most powerful electron acceptors. In recent years it became clear that microorganisms can take advantage of the oxidizing power of these compounds to convert recalcitrant aliphatic and aromatic hydrocarbons. For two unrelated bacterial species, the ‘NC10’ phylum bacterium ‘Candidatus Methylovorans ilumifluorescens’ and the γ-proteobacterial strain HdN1 it has been suggested that under anoxic conditions with nitrate or nitrite, monoxygenases are used for methane and hexadecane oxidation, respectively. No degradation was observed with nitrous oxide. Similarly, “aerobic” pathways for hydrocarbon degradation are employed by (per)chlorate-reducing bacteria, which are known to produce oxygen from chlorite (ClO₃⁻). In the anaerobic methanotroph M. oxyfex, which lacks identifiable enzymes for dinitrogen formation, degradation of methane in the presence of nitrite was directly associated with both oxygen and dinitrogen formation. These findings strongly argue for the role of NO, or an oxygen species derived from it, in the activation reaction of methane. Although oxygen generation elegantly explains the utilization of ‘aerobic’ pathways under anoxic conditions, the underlying mechanism is still elusive. We will discuss the current knowledge on intra-aerobic pathways, their potential presence in other organisms and present candidate enzymes related to quinol-dependent NO reductases (qNORs) that might be involved in the formation of oxygen.

doi:10.1016/j.bbabio.2012.06.406

21P3
The energy sulfur metabolism of the hyperthermophilic bacterium Aquifex aeolicus
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Aquifex aeolicus, a highly thermophilic bacterium, grows at 85 °C with molecular hydrogen as electron donor and molecular oxygen as electron acceptor. A source of sulfur (elemental sulfur, thiosulfate or hydrogen sulfide) is absolutely required for growth. Stimulated by its exceptional physiological properties, we have set out to study its energy metabolism and the proteins involved in the bioenergetic pathways. We have combined tools of proteomics, biochemistry, and physical-chemistry to characterize two high molecular weight membrane-bound protein edifices representing two complete “respirasomes” involved in two distinct respiratory pathways. One superstructure, formed by the stable association of a [NiFe] hydrogenase and a molybdenum sulfur reductase, is responsible for sulfur reduction by H₂ [1]. The second one is a supercomplex involved in oxygen respiration and contains all proteins and complexes required for the electron transfer from H₂S to O₂. It contains the monotonic flavoenzyme sulfide quinone reductase (Sqr), the bc complex and the three-subunit bo3 cytochrome c oxidase [2, 3]. Thus two different energy pathways (sulfur reduction and sulfur oxidation) are organized in this bacterium as supramolecular structures in the membrane. Moreover, additional cytoplasmic enzymes involved in sulfur compound utilization were characterized from A. aeolicus: sulfur oxygenase reductase (SOR), which catalyzes the simultaneous oxidation and reduction of elemental sulfur, in the presence of oxygen [4] and a sulfur transferase SbdP [5] which might function as a sulfur supplier, distributing elemental sulfur to some enzymes of energy sulfur metabolism, and thus optimizing respiration on elemental sulfur [6]. Taking advantage of the complete genome sequence of A. aeolicus, in addition to the experimental data, we propose a model for the energetic optimization of sulfur compounds, integrating known and hypothesized pathways [7].

References

doi:10.1016/j.bbabio.2012.06.407

21P4
Ferredoxin:NADP⁺ oxidoreductase junction with CdSe/ZnS quantum dots — An example of enzymatically active nanohybrids to be used in photosynthesis research
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Ferredoxin:NADP⁺ oxidoreductase (FNR) is a key photosynthetic enzyme. Isoforms of FNR may also play a role in other metabolic pathways [1]. Here, we present the hybrid of FNR and quantum dots (QDs) on semiconductor surfaces. Quantum dots (QD) are colloidal nanoparticles, few to several nanometers in diameter. QDs are mainly built from semiconductor materials (e.g. CdSe, CdTe, ZnS), homogenous or composed of core and shell (e.g. CdSe/ZnS). Due to their nano-size and type of semiconductor material, they have unusual fluorescence properties, giving them advantage over typical fluorophores: (i) narrow emission band of maximum tuned by crystal size, (ii) broad absorption spectrum and large Stokes shift, and (iii) significant resistance for photo-bleaching [2].

Functionalization (conjunction of other molecules to a surface) of QDs and other semiconductor-based structures increases their usefulness. We are showing conjugates created by covalent bonding
to activated carboxyl groups of QDs surface [3], as well as by specific interaction between ZnS and ZnS-specific protein tags [4]. Hybrid structures were characterised by several methods, including chromato-
graphy (for overall size and stability), spectrophotometry and
fluorometry (for luminescent properties and the enzyme activity) as
well as microscopy (for surface topography, size and stability). Conju-
gation did not significantly change fluorescent properties of
QDs. What is of high importance, FNR activity was preserved.
Determined parameters of enzyme kinetics (Km, kcat) indicate that
an active site is not altered, although a substrate binding may be
partially hampered.

Our novel nanohybrids may serve in studies of in vivo/in vitro
localization of FNR or its interaction with other proteins, as well as in
examination of electron flow between QD and redox-active proteins.

Acknowledgement

Experiments were partially performed in NanoFun laboratories,
POIG.02.02.00-00-025/09. Financial support was received from the
National Science Centre (grant no. N202 130039) and the Foundation
for Polish Science, Homing PLUS Programme, co-financed by EU
under POIG.

References

[2] U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke,
J.A. Petros, R.M. O’Regan, M.V. Yezbelyev, Y.V. Simons, M.D.
doi:10.1016/j.bbabio.2012.06.409

21P6

Genetically-encoded ATP biosensor for low temperatures

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We have previously reported genetically-encoded FRET biosen-
sors for ATP, named ATeam [1,2]. ATeam is a powerful tool to monitor
ATP levels inside living mammalian cells with high temporal and
spatial resolutions. One of the major drawbacks of the original
biosensors is that their affinity to ATP is very sensitive to tempera-
ture changes. Although dissociation constant (Kd) of the original biosen-
sor, AT1.03, at 37 °C is 3.3 mM, Kd at 24 °C is less than 0.6 mM, far
down physiological ATP concentrations. This means that at low temperatures
(around 24 °C) the FRET signal of AT1.03 biosensor must be saturated with
the physiological concentrations of ATP, making it difficult to
detect a slight change of ATP levels. Because body temperatures of
many model organisms, such as Caenorhabditis elegans and Drosophila
melanogaster, are the same with ambient temperatures (20–25 °C), it
does not seem that the use of AT1.03 biosensor is suitable for these
organisms or cells from them.

In this study, we constructed mutants of AT1.03 by substituting
amino acid residues at the ATP binding domain, the ϵ subunit of F3F1-
ATPase. We found that one of the mutants (AT1.03NL) showed much
lower affinity to ATP than the original AT1.03; Kd was 2.1 mM at 24 °C
and 1.4 mM at 20 °C. To examine if AT1.03NL is actually effective in
ATP imaging at low temperatures, we expressed AT1.03NL and
AT1.03 in S2 cell, which originated from D. melanogaster and was
cultured at 25 °C. If the FRET signal is saturated, it will not respond
quickly to a metabolic challenge. When AT1.03-expressing cells were
treated with 2-deoxyglucose and oligomycin A, there was a lag before
the FRET signal started to decrease. This suggests that the FRET signal
of AT1.03 is mostly saturated in S2 cells. On the other hand, the FRET
signal of AT1.03NL-expressing cells started to decrease immediately
after addition of inhibitors. Thus, the FRET signal of AT1.03NL is not

doi:10.1016/j.bbabio.2012.06.409