ROS production by respiratory complex II
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A common observation is that the rate of H₂O₂ production is remarkably higher under succinate oxidation than under NADH-linked (glutamate/pyruvate) oxidation in isolated rat brain, heart and skeletal muscle mitochondria. As the H₂O₂ production driven by succinate oxidation is strongly inhibited by the CI inhibitor rotenone, it is generally believed that succinate oxidation produces ROS via a "reverse electron flow" from CI to CI. However, oxidation of submillimolar (physiological) concentrations of succinate can produce H₂O₂ even in the presence of high concentrations of NAD-dependent substrates or NADH, which makes the presumed CI→CI reverse electron flow more thermodynamically unfavorable.

Moreover, although rotenone potently block ROS production driven by succinate oxidation, approximately 10–15% of the initial ROS remains, which cannot be catalyzed by CI-FMNH₂. In addition, this ROS fraction may be significantly higher (1.3–15 times) than the ROS produced by the CI forward reaction. The simpler explanation for the generation of the rotenone-insensitive ROS driven by succinate oxidation is that they are produced by the CI redox components. However, it is surprising to find that CII contribution to ROS production has been mostly disregarded and hence has not been experimentally assessed. In the present study, experiments were designed to undoubtedly establish that CII indeed significantly contributes to the generation of ROS.

doi:10.1016/j.bbabio.2012.06.310

15L4

Functional characterization and regulation of UCP4 expression by adipokinetic hormone in larva and pupa fat body mitochondria from the beetle Zophobas atratus
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Uncoupling protein 4 (UCP4) is the member of the large family of mitochondrial anion transporters that uncouple oxidative phosphorylation [1]. The present study demonstrates for the first time, the molecular identification of a partial Zophobas atratus UCP4 coding sequence and the functional characterization of ZaUCP4 in the mitochondria of larval and pupal fat bodies of the beetle. ZaUCP4 shows a high similarity to predicted insect UCP4 isoforms and known mammalian UCP4s, both at the nucleotide and amino acid sequence levels. Bioenergetic studies unequivocally demonstrate UCP4 activity in mitochondria isolated from larvae and pupae fat body. In resting, non-phosphorylating state 4 respiration ZaUCP4 activity was stimulated by palmitic acid and inhibited by the purine nucleotide GTP. In phosphorylating mitochondria, ZaUCP4 activity decreased the yield of oxidative phosphorylation. ZaUCP4 was immunodetected by using of antibodies raised against human UCP4 as a single 36 kDa band. Because it is known that hormones influence an expression of UCPs, we tested adipokinetic hormone (AKH), analog of mammal glucagon, which mobilizes lipids and carbohydrates from fat body stores [2]. Besides this energy-mobilizing function, AKHs inhibit fat body lipid and protein synthesis. After AKH injection, we observed the decrease in ZaUCP4 expression at the mRNA and protein levels in both developmental stages of the beetle.

Real time analysis, immunological detection and bioenergetic characteristic indicate consistently for the higher expression of UCP4 in the Z. atratus larval fat body compared with the pupal fat body, furthermore ZaUCP4 expression is under hormonal control. The different expression patterns and activity of ZaUCP4 during the larval–pupal transformation indicate an important physiological role for UCP4 in insect fat body development and function during insect metamorphosis. Furthermore, the regulation of UCP4 expression by AKH indicates that it may play a role in maintaining metabolic homeostasis in the insect.

This work was supported by the grant of the Polish Ministry of Science (N N 303291634).

References

doi:10.1016/j.bbabio.2012.06.312