P/12 Interaction of antenna carotenoid and retinal in the light-driven proton pump xanthorhodopsin

Janos K. Lanyi, Sergei P. Balashov
Department of Physiology and Biophysics, University of California, Irvine, USA
E-mail: jklanyi@uci.edu

Xanthorhodopsin is a light-driven proton pump like bacteriorhodopsin, but made more effective for collecting light by its second chromophore, salinixanthin, a carotenoid. Action spectra for transport and fluorescence of the retinal upon excitation of the carotenoid indicate that the carotenoid functions as an antenna to the retinal. The calculated distance and angle of the two chromophores are ca. 11 Å and 59°. As expected from their proximity, the carotenoid and the retinal closely interact. Tight binding of the carotenoid, as indicated by its sharpened vibration bands and intense induced circular dichroism in the visible, is removed by removal of the retinal, and restored upon reconstitution with retinal or retinal analogues. The influence of the retinal on the carotenoid depends mainly on steric interaction. Borohydride reduction of the retinal decreases spectral overlap and eliminates energy migration, and as expected, the fluorescence of the carotenoid increases and its excited state lifetime becomes longer. Studies of this antenna system, much simpler than photosynthetic complexes, may reveal fundamental features of excited-state energy migration.

doi:10.1016/j.bbabio.2008.05.024

P/13 Sites of generation of reactive oxygen species in brain tissue

Alexei P. Kudin[a], Dominika Malinska[b], Wolfram S. Kunz[a]
[a]*Division of Neurochemistry, Department of Epileptology, University of Bonn Medical Center, Bonn, Germany*
[b]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*
E-mail: wolfram.kunz@ukb.uni-bonn.de

Reactive oxygen species (ROS) have been widely implicated in the pathogenesis of various neurological diseases and aging. But the exact sites of ROS generation in brain tissue remained so far elusive. Here, we provide direct experimental evidence that about 50% of maximal ROS generation in brain homogenates can be attributed to mitochondrial respiratory chain complex I, about 35% to complex III and about 15% to extramitochondrial sources. Applying quantitative methods for H_2O_2 and superoxide detection we observed in different preparations from human, rat and mouse brain (digitonin-permeabilized tissue homogenates, isolated mitochondria and purified submitochondrial particles) a linear relationship between oxygen consumption rate and maximal rate of ROS generation with succinate as mitochondrial substrate. This quantitative relationship and investigations of digitonin-permeabilized brain homogenates indicate, that in brain tissue mitochondrial respiratory chain contributes to about 85% of ROS formation and that under conditions of oxygen saturation about 1% of the corresponding respiratory chain electron flow is re-directed to form superoxide. Since we observed in mouse and rat brain mitochondria a unique dependency of mitochondrial H_2O_2 production on mitochondrial NAD-redox state, we substantiated previous evidence that the FMN moiety of complex I is the major donor of electrons for the single electron reduction of molecular oxygen.

doi:10.1016/j.bbabio.2008.05.025

P/14 The Bcl-2 family and mitochondria: In sickness and in health

R.J. Youle
Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, NIH, Bethesda MD, USA
E-mail: youler@ninds.nih.gov

Bax and/or Bak, two pro-apoptotic members of the Bcl-2 family, are required for the normal program of cell death during mammalian development. Early during apoptosis, as an essential step in cell death promotion, Bax translocates from the cytosol to mitochondria almost simultaneously with a dramatic fragmentation of the normal mitochondrial network. Subsequent to translocation to the outer mitochondrial membrane, Bax and Bak coalesce into foci on the surface of mitochondria at sites that subsequently constrict and become sites of mitochondrial fission. The mitochondrial fission and fusion machinery is comprised of several proteins including dynamin related protein 1 (Drp1) and mitofusin 2 (Mfn2) both of which colocalize with Bax in foci at prospective mitochondria fission sites. Inhibition of mitochondrial fission inhibits apoptosis whereas in healthy cells Bax or Bak is required for the normal rate of mitochondrial fusion. How Bax and Bak intersect the mitochondrial fission and fusion machinery is under investigation. Bax can bind to Mfn2 and to Endophilin B1, two proteins normally involved in regulating mitochondrial morphology in healthy cells. How Bax regulates the activity of these Mfn2 and Endophlin B1 to impact mitochondrial activity will be discussed.

doi:10.1016/j.bbabio.2008.05.026

P/15 Mitochondrial Ca^{2+} homeostasis in cell life and death

R. Rizzuto, P. Pinton, P. Aguiari, T. Blom, F. Celsi, D. De Stefani, C. Fotino, S. Leo, M. Marchi, A. Rimessi, A. Romagnoli, R. Siviero, E. Zecchini
University of Ferrera, Italy
E-mail: rzr@dns.unife.it

Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high [Ca^{2+}] microdomains generated by the opening of ER Ca^{2+} channels. These rapid [Ca^{2+}] changes stimulate Ca^{2+}-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. At the same time, Ca^{2+} sensitizes to cell death mediators acting on mitochondria, such as ceramide. In agreement with this notion, we demonstrated that Bcl-2 reduces the state of filling of ER Ca^{2+} stores, and this alteration is effective in reducing the sensitivity to various apoptotic challenges. I will here review the latest data of the lab focusing on: (1) The effect on mitochondrial Ca^{2+} homeostasis of other signalling pathways involved in apoptosis (Akt, FHIT), (2) The signalling route that links oxidative stress to the activation of p66shc, an isoform of a growth factor adapter acting as apoptotic inducer. We demonstrated that PKCβ, activated by the oxidative challenge, induces p66shc phosphorylation, with ensuing alteration of mitochondrial structure and function. We also showed that this route is involved also in adipose differentiation of muscle-derived precursors, highlighting a novel process of utmost interest in pathophysiological conditions. (3) The molecular elements of the mitochondria-ER Ca^{2+} connection. I will discuss the role of VDAC in rapidly channelling Ca^{2+} through the mitochondrial outer membrane and the specific functions of the various VDAC isoforms.

doi:10.1016/j.bbabio.2008.05.027