Mitochondrial and cell-surface F$_0$F$_1$ATP synthase in innate and acquired cardioprotection

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Abstract Mitochondria are central to heart function and dysfunction, and the pathways activated by different cardioprotective interventions mostly converge on mitochondria. In a context of perspectives in innate and acquired cardioprotection, we review some recent advances in F$_0$F$_1$ATP synthase structure/function and regulation in cardiac cells. We focus on three topics regarding the mitochondrial F$_0$F$_1$ATP synthase and the plasma membrane enzyme, i.e.: i) the crucial role of cardiac mitochondrial F$_0$F$_1$ATP synthase regulation by the inhibitory protein IF$_1$ in heart preconditioning strategies; ii) the structure and function of mitochondrial F$_0$F$_1$ATP synthase oligomers in mammalian myocardium as possible endogenous factors of mitochondria resistance to ischemic insult; iii) the external location and characterization of plasma membrane F$_0$F$_1$ ATP synthase in search for possible actors of its regulation, such as IF$_1$ and calmodulin, at cell surface.

Keywords Mitochondrial F$_0$F$_1$ATP synthase · Inhibitory protein IF$_1$ · Supra-molecular complexes · F$_0$F$_1$ATP synthase oligomers · Cell surface F$_0$F$_1$ ATP synthase · Calmodulin · Cardioprotection

Introduction

Mitochondria have a central role in cardiac function and dysfunctions, as they modulate energetics, reactive radical biology, calcium homeostasis and apoptosis, as well as orchestrate nuclear regulation, metabolic pathways and cell survival programs via retrograde signalling. In normal cardiac tissue ATP produced by oxidative phosphorylation (OXPHOS) is over 90% and is used preferentially to support myocyte contractile activity. Acute pump failure in ischemia increases ATP demand in excess of ATP supply, thus some established therapeutic strategies are interventions that reduce energy demands during ischemia (Marín-García and Goldenthal 2008). F$_0$F$_1$ ATP synthase (OXPHOS complex V) in the inner mitochondrial membrane synthesizes ATP using the H$^+$ electrochemical gradient (m$\Delta$Ψ) generated by the respiratory chain. This is a reversible enzyme that can also hydrolyse ATP in a reaction coupled to a proton transport out of the mitochondrial matrix thus sustaining m$\Delta$Ψ. Electron microscopy studies on native mitochondrial membranes from different sources, including mammalian heart (Thomas et al. 2008; Strauss et al. 2008) have recently evidenced that F$_0$F$_1$ ATP synthase is present as dimers associated to form long row of oligomers. These structures are now considered as the physiological state of the enzyme in membrane, although F$_0$F$_1$ ATP synthase is commonly isolated as a functional monomer. This latter is composed of a soluble catalytic F$_1$ part connected by two stalks with a membrane-embedded F$_0$ part, which functions as a proton channel. The mammalian enzyme is built of at least 16 different subunits, F$_1$: $\alpha_3\beta_3\gamma\delta\epsilon$ + IF$_1$, F$_0$: a, b, c$_{10}$, d, e, f, g, F$_6$, A6L, OSCP, (factor B). (Wittig and Schagger 2008; Belogrudov 2008). Our knowledge of the enzyme membrane domain is not complete and the structures of the so-called “minor” subunits (e, f, g and A6L), as well as...
those of the more loosely associated proteins DAPIT and 6.8-kDa proteolipid, are not known either. These proteins are unlikely to have a role directly in ATP synthesis, but they appear to influence variously the oligomeric state of the enzyme in the mitochondrial inner membrane (Chen et al. 2007; Meyer et al. 2007). IF1, a basic protein of 84 amino acids in length, is a non-competitive inhibitor that reversibly binds to F0F1ATPsynthase with 1:1 stoichiometry in a \( \alpha-\beta \) interface (Gledhill et al. 2007). Binding requires ATP hydrolysis and is favoured by low pH and \( \Delta \psi \). The restoration of \( \Delta \psi \) favouring ATP synthesis displaces IF1 from its inhibitory site (Green and Grover 2000).

Many reports from several laboratories in recent years have concerned the location and function of the ATP synthase complex or its component subunits on the external surface of the plasma membrane of various mammalian cell types, including vascular endothelial cells, hepatocytes, adipocytes and tumor cells. F0F1 components, most usually \( \beta \) subunit, have been identified as cell-surface receptors for multiple ligands in studies carried out on angiogenesis, tumor proliferation/toxicity, lipid/lipoprotein metabolism, immune recognition of tumors and hypertension (Champagne et al. 2006; Chi and Pizzo 2006a, b; Mangiullo et al. 2008).

**Transient inhibition of cardiac mitochondrial**

**\( F_{0}F_{1} \)ATP synthase in preconditioning**

It is widely accepted that activation of endogenous protective responses by stimuli applied immediately before ischemia (ischemic preconditioning) or at reperfusion (postconditioning), that persist even after the initiating stimulus is removed, increases myocardial tolerance to ischemia-reperfusion injury (Gross and Auchampach 2007; Bolli 2007; Yellon and Opie 2006). Insights into mechanisms underlying these innate cardio-protective responses have resulted in the exploration of novel therapeutic avenues (Murphy and Steenbergen 2008). The pathways activated by ischemic and pharmacological preconditioning (PC), as well as by postconditioning mostly converge on mitochondria (Marin-Garcia and Goldenthal 2004; Correa et al. 2008).

It is now well known that PC induces bioenergetic tolerance and thereby remodels energy metabolism that is crucial for post-ischemic recovery of the heart. PC-hearts have less anaerobic glycolysis during the sustained period of ischemia than non-PC-hearts and the rate of ATP consumption is slower. Thus ATP levels fall more slowly, despite the fact that PC-hearts start with a lower ATP (Murry et al. 1990; Steenbergen et al. 1993; Fralix et al. 1993). An early hypothesis to account for the reduced ATP breakdown was that PC might inhibit ATP hydrolysis by a reverse mode of the mitochondrial \( F_{0}F_{1} \)ATP synthase (\( mtF_{0}F_{1} \), consistent with the finding that during ischemia the \( \Delta \psi \) depolarized to a greater extent in PC-hearts (Ylitalo et al. 2000). It has been reported that as much as 35–50% of the ATP generated by glycolysis during ischemia is consumed by the reverse-mode of the \( mtF_{0}F_{1} \) (Harris and Das 1991; Di Lisa et al. 1995; Grover et al. 2004). Several data suggest that during ischemia the \( mtF_{0}F_{1} \) inhibitory subunit \( IF1 \) binds to the enzyme and inhibits the ATPase activity, thus limiting ATP decline (Rouslin 1983; Rouslin and Broge 1996; Campanella et al. 2008). Then, it was proposed that PC promoted earlier binding of \( IF1 \) to \( mtF_{0}F_{1} \) (Ylitalo et al. 2000), but this was severely criticized (Green et al. 1998; Vander Heide et al. 1996). Other mitochondrial mechanisms were suggested including opening of the mito\( K_{ATP} \) channel that was proposed to prevent the matrix condensation caused by ischemia thereby decreasing ATP breakdown (Garlid et al. 1997). In the last few years ischemic PC (Ala-Rami et al. 2003; Penna et al. 2004; Di Pancrazio et al. 2004) and diazoxide treatment (Dzeja et al. 2003; Contessi et al. 2004; Comelli et al. 2007) have been reported to enhance \( IF1 \) binding to \( mtF_{0}F_{1} \), thereby renewing the emphasis of the early hypothesis of the Hassinen group that the \( mtF_{0}F_{1} \) down-modulation by PC is one important component of energy preservation in ischemic myocardium.

A review of our data supporting such hypothesis is presented below (Fig. 1b). In our opinion this hypothesis is reinforced by the recent observation that the opening probability of the mito\( K_{ATP} \) channel from cardiac mitochondria reconstituted into planar lipid bilayers is decreased by acidification (Bednarkzyk et al. 2008). As the condition used by the authors mimic ischemic acidosis, we infer that this may be the case even in vivo diminishing the relevance of the Garlid mechanism in reducing the energy waste.

We first studied a model of in vivo ischemic PC on anaesthetized open-chest goat heart, able to prevent coronary reactive hyperemia (CRH). CRH is the increase in flow that follows a very brief coronary occlusion caused primarily by the release of vasoactive compounds from ischemic myocardium (Penna et al. 2004; Di Pancrazio et al. 2004). Functional and electrophoretic analyses on heart biopsies proved that \( mtF_{0}F_{1} \) is inhibited by \( IF1 \) during PC, whereas it is up-modulated through release of \( IF1 \) upon CRH. Evidence that PC-elicited inhibition is slowly reversible points to an important role in ATP preservation during prolonged ischemia. Furthermore, according to our results the only significant modulator of the enzyme activity is \( IF1 \), as we observed a close inverse correlation between enzyme activity and \( IF1 \) content by plotting the activity vs electrophoretic data (obtained by detergent extraction of single heart biopsy samples and quantitative...