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Redox-regulation of mitochondrial ATP synthase

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Abstract

Reversible cysteine oxidative post-translational modifications (Ox-PTMs) represent an important mechanism to regulate protein structure and function. In mitochondria, redox-reactions can modulate components of the electron transport chain (ETC), the F_1F_O-ATP synthase complex and other matrix proteins/enzymes. Emerging evidence has linked Ox-PTMs to mitochondrial dysfunction and heart failure, highlighting some potential therapeutic avenues. Ox-PTMs can modify a variety of amino acid residues, including cysteine, and have the potential to modulate the function of a large number of proteins. Among this group, there is a selected subset of amino acid residues that can function as redox-switches. These unique sites are proposed to monitor the cell's oxidative balance through their response to the various Ox-PTMs. In this review, the role of Ox-PTMs in the regulation of the F_1F_O-ATP synthase complex is discussed in the context of heart failure and its possible clinical treatment.

Introduction

Reactive oxygen/nitrogen species (ROS/RNS) have a dual nature in the cell. Excess generation of ROS/RNS contributes to the development and progression of many diseases, including cardiovascular disease; however, increasing evidence suggests that lower concentrations of ROS/RNS can contribute to cellular signaling (Valko et al. 2007). The mitochondria produce the majority of cellular ATP via the concerted actions of the electron transport chain (ETC) and the F_1F_O-ATP synthase (Boyer 1997). The ETC is also a primary source of ROS/RNS production in the mitochondria which has been found to impact the F_1F_O-ATP synthase complex. There is considerable knowledge about the structure and function of F_1F_O-ATP synthase and its subunits (Ackerman and Tzagoloff 2005, Feniouk and Yoshida 2008, Pedersen 2007). In the last few years, a number of phosphorylated amino acid residues have been identified (Agnetti et al. 2010, Arrell et al. 2006, Deng et al. 2011) and, in some cases, shown to alter the function of the F_1F_O-ATP synthase complex (Kane et al. 2010). Until recently, little was known regarding the Ox-PTM-dependent regulation of the ATP synthase subunits. Advances in PTM-specific proteomic methods (antibody and mass spectrometry based) for Ox-PTMs of cysteine residues have allowed for the detection

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of a surprising number of Ox-PTMs in subunits of F_1F_O-ATP synthase (Kane et al. 2009, Nadtochiy et al. 2012, Rexroth et al. 2012, Sun et al. 2007, Wang et al. 2011). Mitochondrial Ox-PTMs have recently been associated with cardiac resynchronization therapy (CRT, also called atrial-synchronized biventricular pacing, Wang et al. 2011). CRT is a therapeutic technique which involves electrical pacing from two locations in the heart which coordinates the contraction of the heart and improves the efficiency of each contraction (Kass 2009). Among the many targets of this treatment, CRT has been found to significantly impact mitochondrial function (Agnetti et al. 2010). In this mini-review, we briefly discuss some recent findings involving intra-mitochondria redox-signaling with emphasis on cysteine Ox-PTMs of specific F_1F_O-ATP synthase subunits and the functional implications in the context of an animal model of heart failure and CRT.

Cysteine Ox-PTM of mitochondria ATP synthase

One aspect of redox-regulation can occur via the selective covalent modification of proteins in response to changes in local redox-status within a cell. The thiol chemistry of the cysteine residue's side chain is uniquely suited to this task as it can adopt multiple oxidation states (Forman et al. 2004; Winterbourn and Hampton 2008). However, not all cysteine residues respond equally to changes in local redox-potential. Some cysteine residues are more reactive than others, and this reactivity largely depends on the exposure and pK_a of each thiol. The pKa of a cysteine residue can be influenced significantly by the makeup of the surrounding amino acid residues. The presence of polar or positively charged amino acids can stabilize the thiolate form through electrostatic interactions and decrease the pKa to as low as 3.5 (Salsbury et al. 2008). The lower the pKa, the greater the proportion of the time the thiol will spend deprotonated in the thiolate form, making it more reactive to oxidative modification (Winterbourn and Metodiewa 1999). ROS and RNS can target highly reactive thiols to yield a number of species (Figure 1). These include reversible Ox-PTMs such as intra-molecular and mixed disulfides, sulfenic acid intermediates and S-nitrosothiols as well as irreversible oxidative modifications (e.g. sulfonic acid) (Hurd et al. 2005a, b; Janssen-Heininger et al. 2008). In mammals, ATP synthase was not previously known to conduct any redox-related functions; however, the regulation of the chloroplast ATP synthase in plants by the redox-state has been well established (Bald et al. 2001). Specifically, the ATP hydrolytic activity of chloroplast ATP synthase is regulated by the formation and reduction of a disulfide bond located in the γ-subunit. This change in redox-state does not affect ATP binding rates to the catalytic site(s) or torque for rotation, but instead causes a long pause in the catalytic process due to ADP inhibition (Kim et al. 2011). Introducing this redox-sensitive amino acid sequence from the plant into the yeast γ-subunit has been found to cause a defect in oxidative phosphorylation (Shen et al. 2008).

In mammalian cells, the chloroplast redox-sensitive γ-subunit region is not present; however, mitochondrial F_1F_O-ATP synthase complex may be a "hot spot" for Ox-PTMs. Interestingly, Garcia *et al*. have reported that the F_1F_O -ATP synthase α-subunit is S-glutathionylated under oxidative stress in rat brain or liver mitochondria (Garcia et al. 2010). Under these conditions, S-glutathionylation of α-subunit led to a substantial decrease in ATPase activity. This process was reversed by the supplementation of mitochondria with respiratory substrates, which indicated these substrates were key regulators of mitochondrial redox-status by maintaining mitochondrial NADPH levels (Garcia et al. 2010). Similar results were also observed in acetylcholine-treated aortic tissue and in transgenic mouse hearts that over-expressed iNOS, where the α-subunit of ATP synthase was identified as the major target for S-glutathionylation (West et al. 2006). Furthermore, Sun *et al*. reported that mouse hearts preconditioned with GSNO treatment led to the S-nitrosation (also known as S-nitrosylation) of the mitochondrial ATP synthase α-subunit which resulted in a decrease in its ATP hydrolytic activity (Sun et al. 2007). These authors concluded that increased S-

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nitrosation decreased ATP synthase activity and suggested that this inhibition may reduce the consumption of ATP during myocardial ischemia by reversing the mode of the ATP synthase (Sun et al. 2007). This could preserve the cellular concentration of ATP reducing the mitochondrial membrane potential ($\Delta\psi$) resulting in a diminished driving force for Ca^{2+} uptake into the mitochondrial matrix (Sun and Murphy, 2010). This change in energy consumption would ultimately result in a protective phenotype. However, there are many proteins within the mitochondria that can undergo S-nitrosation in addition to ATP synthase. In fact, Murray *et al*. have shown 83 cysteines on 60 proteins that could be modified by GSNO treatment in isolated mitochondria, including cysteine 294 of ATP synthase α-subunit which was also observed in an earlier study (Murray et al. 2010, Sun et al. 2007).

Redox regulation of ATP synthase in heart failure

Heart failure (HF) patients who develop a regional conduction delay that results in discoordinate contraction have exacerbated symptoms and a more negative ultimate prognosis compared to patients who avoid a conduction delay. In the last decade, CRT has been introduced as a clinical treatment for these patients, improving heart function, clinical symptoms and survival (Kass 2009). Early clinical studies demonstrated that CRT improved chamber energetic efficiency; a reduction in wasted chamber work that occurs because the contraction is re-timed to happen synchronously in both sides of the myocardium (Chakir et al. 2011). In an animal model of dyssynchronous heart failure (DHF), the mitochondrial ATP synthase activity was significantly reduced which correlated to phosphorylation of ATP synthase (Agnetti et al. 2010). Recently we have shown that this inhibition was not solely linked to phosphorylation but also to the site-specific Ox-PTMs of mitochondrial F_1F_O -ATP synthase subunits (Wang et al. 2011). As shown in Figure 2, two cysteine disulfide bonds were identified between ATP synthase α- and γ-subunit and between α-subunits in heart failure. The first disulfide links cysteine 294 of the α-subunit to cysteine 103 of the γ-subunit. In addition, the ATP synthase α-subunit can be S-glutathionylated and S-nitrosated (both occur at cysteine 294) in failing hearts. The extent of cysteine cross-linking (including an intermolecular disulfide bond and a mixed disulfide bond with S-glutathionylation) is negatively correlated with ATP synthase hydrolytic activity. It is possible that disulfide bond formation within the ATP synthase complex can cause profound conformational changes leading to loss of function. S-glutathionylation adds a bulky, negatively charged group to the same residue which has the potential to disrupt nucleotide binding (Chen et al. 2010) and both modifications could lead to decreased ATP synthase activity. Overall, these findings suggest that cysteine cross-linking within ATP synthase contributes to mitochondrial dysfunction in heart failure. In CRT, the cysteine cross-linking has been found to be displaced by S-nitrosation at the same cysteine residues. Importantly, this change has been found to recover ATP synthase activity in CRT treated dogs.

Cysteine Ox-PTMs are dependent on the redox-status and antioxidant capacity of the cell (Valko 2007). Our group has shown that CRT significantly increases the content of peroxiredoxin 3 (Prx3), a mitochondrial H_2O_2 scavenger, which is maintained in the reduced state by thioredoxin (Trx) (Agnettti et al. 2010). We have also found that the quantity and activity of Trx1 was decreased in DHF, but restored in CRT (preliminary data). Taken together, this suggests that CRT improves antioxidant defenses, in particular, the Trx/Prx pathway. Enhanced ROS scavenging might also prevent NOS uncoupling and preserve the physiological nitric oxide (NO) signaling mechanisms, including S-nitrosation. Chen and colleagues have shown that the increased production of ROS induces S-glutathionylation of endothelial NOS (eNOS) which in turn uncouples the enzyme and converts eNOS from its classical NO synthase function to that of an NADPH-dependent oxidase generating superoxide (Chen et al. 2010). This situation could occur in DHF, where increased oxidative stress would result in S-glutathionylation and subsequent uncoupling of eNOS, resulting in

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reduced NO production even with further oxidative stress. This would contribute to the decrease in S-nitrosation and the increase in S-glutathionylation of ATP synthase and likely other, yet to be defined, mitochondrial proteins. Thus, the interplay between the various Ox-PTMs of ATP synthase in DHF and CRT may reflect a broader cellular or mitochondrial phenomenon, in which selective cysteine residues in key proteins act as redox-switches. Identification of these redox-switches will provide insights into the molecular basis of DHF and how to modulate their function to mimic CRT.

Specific cysteines function as redox-switches

The finding that multiple Ox-PTMs can occur at cysteine 294 in the ATP synthase α subunit, each competing with another for occupancy of this residue, is strong evidence for this amino acid's role as a redox-sensor (Figure 2). Based on the x-ray crystal structure (Gibbons et al. 2007), Cys294 of ATP synthase α subunit is located on the surface of ATP synthase and surrounded by several basic amino acid residues. It is likely to be deprotonated at physiological pH, making it a good candidate for oxidant attack which allows it to detect changes in the redox-status of the local cellular environment and regulate ATP synthase function. Specifically, under the severe oxidative stress present in heart failure, cysteine 294 could be cross-linked to form an intermolecular disulfide bond or be covalently modified by reduced glutathione to form a mixed disulfide bond. Disulfide bond formation would severely decrease ATPase activity and could eventually induce mitochondrial dysfunction. With CRT, antioxidant protective systems are enhanced which results in the disulfide bond being reversed, and significantly improved ATPase activity. Thus, this single modifiable cysteine in the ATP synthase complex can act as a redox-modulator of cellular ATP concentration. Most likely, other key mitochondrial proteins are also able to sense the redox-potential of the local environment and act as molecular "switches", regulating mitochondrial function in a coordinated manner.

Future perspectives

There are a number of areas for possible future study. It has been proposed that specific Ox-PTMs contribute to functional defects in DHF and that modulation of these target sites contributes to the beneficial effects of CRT. A complete view of the therapeutic potential of CRT requires an understanding of how changes in the PTM status of principal proteins involved in ATP production contribute to overcoming the energetic deficiencies in DHF. It will be important to quantitatively analyze the proportion of each Ox-PTM in the different animal models. The recently developed cysteine reactive tandem mass tag (CysTMT, Thermo Scientific) switch assay, which can identify and quantify the extent of modification by mass spectrometry in up to six samples simultaneously, is a promising approach for this purpose (Murray et al. 2012). Additionally, it will be important to identify and characterize critical and highly reactive redox-switches that play a role in the cellular response to ROS and RNS. It will also be key to understand the relationship between protein Ox-PTMs status, ROS levels, NO levels, and cardioprotection in the context of heart failure. As discussed, S-nitrosation of ATP synthase can be beneficial, while other Ox-PTMs are detrimental. Understanding the underlying mechanisms controlling the S-nitrosation of ATP synthase in CRT and the relationship between S-nitrosation and disulfide bond formation will provide valuable insight. Finally, the cross-talk between redox and other signaling cascades, such as phosphorylation, will shed light on how the cell's defense mechanisms are coordinated. It is an appealing concept that phosphorylation (or other PTMs) and redox-induced PTM would allow for fast and coordinated mitochondrial responses to changes in the local cellular environment. This level of responsiveness would be advantageous for the myocyte to adapt and survive.

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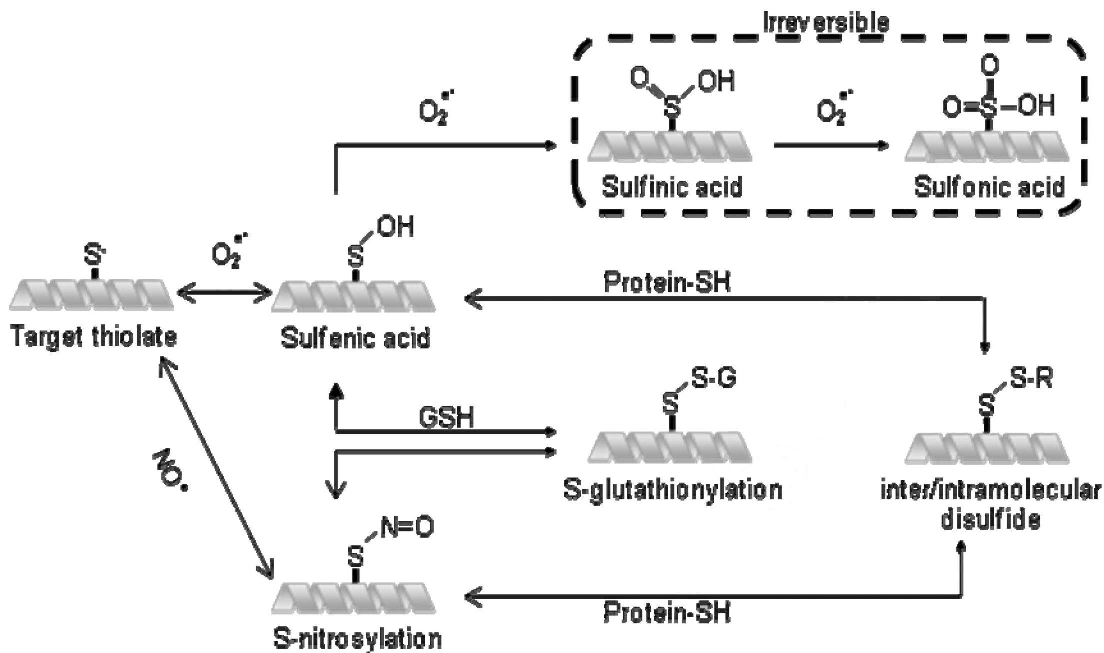

Figure1.
Reversible and irreversible cysteine Ox-PTMs under physiological and pathological conditions. Protein cysteine thiols (R-SH) can undergo a broad range of chemical reactions. In the presence of hydrogen peroxide or other reactive oxygen species (ROS), protein thiol groups can be oxidized to sulfenic acid, which is very unstable and reactive. Sulfenic acid can form a disulfide bond with another protein's free thiol, or form a mixed disulfide with glutathione, which is referred to as glutathionylation. Disulfides and glutathione adducts can be reduced back to the thiol form by thioredoxin (Trx) and glutaredoxin (Grx), respectively. Under pathological condition, Sulfenic acid can be further oxidized to sulfinic acid and sulfonic acid, which are generally regarded as irreversible. Thiol groups can also be oxidized by reactive nitrogen species (RNS) and form S-nitrosothiols. S-nitrosothiols can nitrosylate other cysteine thiols by a transnitrosylation reaction or they can be denitrosylated by reduced Trx.

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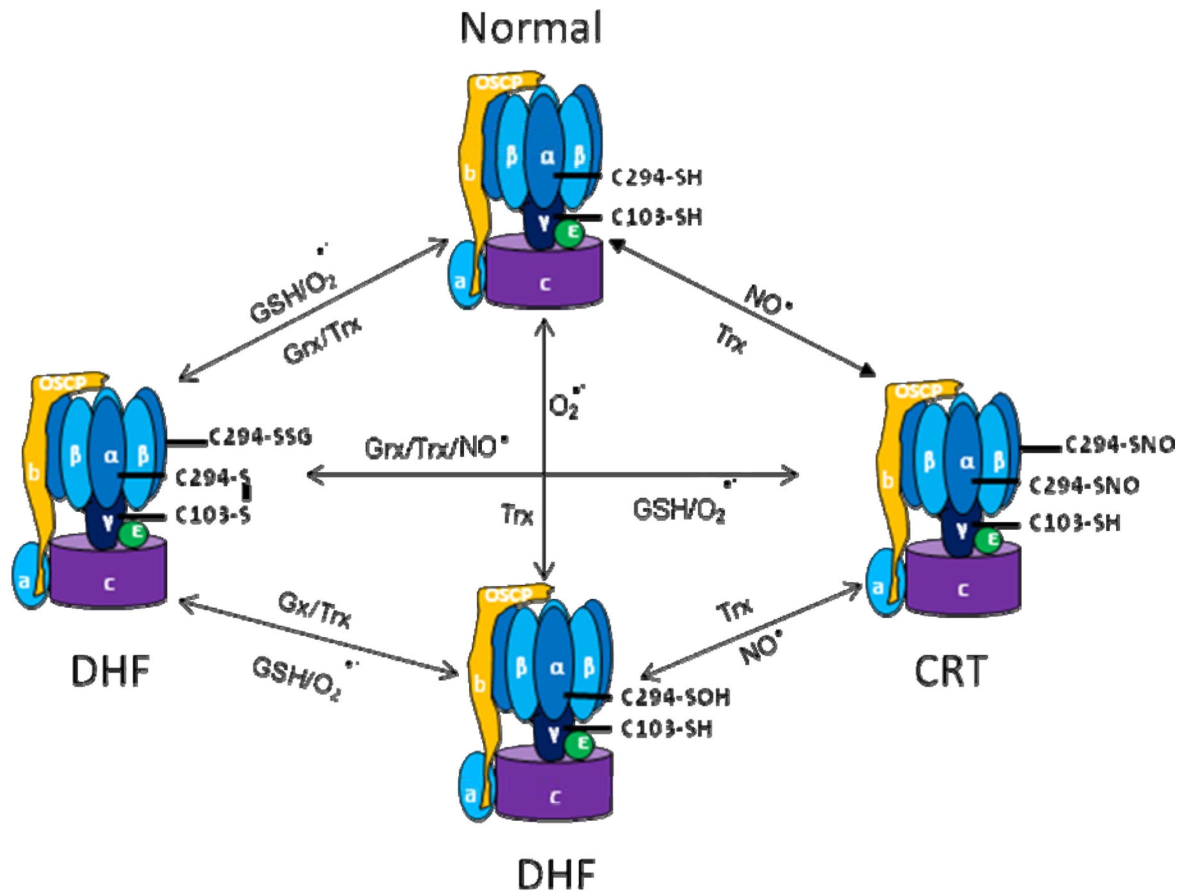

Figure 2.

ATP synthase α subunit Cys 294 functions as a redox swithche. In DHF, Cys294 of the α-subunit is oxidized to sulfenic acid with increasing ROS production. As DHF progresses, the sulfenic acid can be further oxidized to form inter-protein disulfide bonds between Cys294 of α-subunits and between Cys294 and Cys103 of the γ-subunit or form a mixed disulfide bonds with GSSG (S-glutathionylation). Disulfide bond formation inhibits its ATP synthesis activity, leading to mitochondrial dysfunction. CRT treatment activates cellular anti-oxidative defense pathways (probably the Grx/Prx/Trx system) which reverses Cys cross-linking and increases S-nitrosylation of ATP synthase at Cys294 of the α-subunit, along with recovered ATPase activity.