Critical Review

Cardiolipin Drives Cytochrome c Proapoptotic and Antiapoptotic Actions

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Summary

Cytochrome c (cyt c) is pivotal in mitochondrial respiration and apoptosis. The heme-Fe-atom of native hexacoordinated horse heart cytochrome c (hhcyt) displays a very low reactivity toward ligands and does not exhibit catalytic properties. However, on interaction with cardiolipin (CL), hhcyt changes its tertiary structure disrupting the heme-Fe-Met80 distal bond. The CL-hhcyt complex displays a very low midpoint potential, out of the range required for its physiological role, binds CO and NO with high affinity, facilitates peroxynitrite isomerization to NO3, and displays peroxidase activity. As a whole, the CL-hhcyt complex could play either proapoptotic effects, catalyzing lipid peroxidation and the subsequent hhcyt release into the cytoplasm, or antiapoptotic actions, such as scavenging peroxynitrite (i.e., protecting the mitochondrial from reactive nitrogen and oxygen species), and binding of CO and NO (i.e., inhibiting lipid peroxidation and hhcyt traslocation). Here, the CL-driven allosteric modulation of hhcyt properties is reviewed, highlighting proapoptotic and antiapoptotic actions. © 2011 IUBMB

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Abbreviations CL, cardiolipin; cyt c, cytochrome c; cyt-cFe(II), ferrous cytochrome c; CL-cyt c, CL-bound cytochrome c; hhcyt, horse heart cytochrome c; hhcyt-Fe(II), ferrous horse heart cytochrome c; hhcyt-Fe(III), ferrous horse heart cytochrome c; CL-hhcyt, CL-bound horse heart cytochrome c; CL-hhcyt-Fe(III), cardiolipin-ferric horse heart cytochrome c complex; CL-hhcyt-Fe(II), cardiolipin-bound ferric horse heart cytochrome c complex; Mb, myoglobin; Mb-Fe(II), ferrous myoglobin.

Eukaryotic cytochromes c (cyt c) are small water-soluble globular heme-proteins that are located within the compartment delimited by the inner and outer mitochondrial membranes playing a pivotal role in mitochondrial respiration and apoptosis (1–5).

In mitochondria, cyt c is located between the inner and the outer membrane and functions to transfer electrons from Complex III (UQH2-cyt reductase) to Complex IV (cyt oxidase) of the respiratory chain. It mediates electron transfer through the heme group, which switches between the reduced ferrous form (Fe(II)) and the oxidized ferric (Fe(III)) state. Moreover, in healthy cells, cyt c inhibits reactive oxygen species formation, thus preventing cell oxidative stress (3, 6–8).

Cyt c displays also a central apoptotic role. Cyt c release into the cytosol is particularly associated with activation of the intrinsic pathway, which responds to intracellular stimuli such as DNA damage and oncogene activation. Multiple cytosolic and mitochondrial proteins regulate the mitochondrial pathway of cell death. Members of the Bcl-2 family regulate events upstream of mitochondria, weighing the prosurvival signals against the stress/damage signals. If the latter prevail, the mitochondrial membrane is permeabilized, leading to deterioration of the bioenergetic functions of mitochondria, overproduction of reactive oxygen species, as well as to the release of cyt c into the cytosol. Once in the cytosol, in the presence of ATP (and more efficiently in the presence of deoxyATP), cyt c mediates the allosteric activation and hepta-oligomerization of the adapter molecule apoptosis-protease activating factor-1, generating the complex known as apoptosome. Each apoptosome can
rational to apoptosis. In fact, upon CL binding, cyt is pivotal for switching cyt into the mitochondrial inner membranes bound to cardiolipin (CL). CL-cyt forms a complex delimited by the inner and the outer membranes or localized biochemical and morphological features of apoptosis. CL-cyt can recruit caspase 3, which mediates the biochemical and morphological features of apoptosis. These events induce the catalytic maturation of caspase 3, which mediates the biochemical and morphological features of apoptosis.

Figure 1. The multiple functions of cyt. In the mitochondria, cyt mediates electron transfer from Complex III (III) to Complex IV (IV). Mitochondrial cyt is placed within the compartment delimited by the inner and the outer membranes or localized into the mitochondrial inner membranes bound to cardiolipin (CL). CL-cyt binds CO and NO with high affinity and facilitates peroxynitrite conversion to NO3− (antiapoptotic action). CL-cyt displays peroxidase activity, and CL being a favorable substrate, this pathway allows cyt to release into the cytoplasm. Once in the cytosol, in the presence of deoxyATP (dATP), cyt mediates the activation of the adaptor molecule apoptosis-protease activating factor-1 (Apaf-1), generating the apoptosome. Apoptosome can recruit caspase 9 favoring proteinase activation. These events induce the catalytic maturation of caspase 3, which mediates the biochemical and morphological features of apoptosis.

At least 15% of mitochondrial cyt is bound to cardiolipin (CL), an unusual lipid largely confined to the inner mitochondrial membrane. The interaction with CL is pivotal for switching cyt function(s) from mitochondrial respiration to apoptosis. In fact, upon CL binding, cyt has been shown (i) to change its tertiary structure disrupting the heme-Fe-Met80 distal bond (the proximal axial ligand being His) and, in some cases, to vary the spin state of the metal (4, 14, 18–22), (ii) to reduce drastically the midpoint potential out of the range required for its role in the respiratory chain (17), (iii) to display peroxidase activity, using CL as a favorable substrate (15, 23–26), (iv) to bind CO and NO with high affinity (27, 28), and (v) to facilitate peroxynitrite scavenging (i.e., conversion to NO3−) (29). All these effects suggest that CL-bound cyt (CL-cyt) could play either proapoptotic effects, catalyzing lipid peroxidation, and cyt release into the cytoplasm, or antiapoptotic actions, scavenging peroxynitrite (i.e., protecting the mitochondrion from reactive nitrogen and oxygen species), and binding CO and NO (i.e., inhibiting lipid peroxidation and cyt translocation). Here, the CL-driven cyt proapoptotic and antiapoptotic actions are reviewed.

**HOW CARDIOLIPIN BINDS TO HORSE HEART CYTOCHROME C?**

CL, which constitutes about 20% of total lipids of the mitochondrial membrane, is synthesized in the mitochondrion and possesses a unique structure, being composed of four (instead of two, as in most lipids) acyl chains (20, 30).

To the authors’ knowledge, no crystal structures are available for the CL-cyt complex, thus the proposed binding modes are based on available experimental data and molecular modeling investigations. Although CL/cyt recognition is uncertain and still matter of controversy, both regions considered by molecular modeling studies provide suitable sites for CL binding (18, 20, 21).

The first model to be proposed suggests that on CL binding to horse heart cyt (hhcyt), one acyl chain of CL protrudes into the protein interior through the hydrophobic channel located close to the Asn52 residue. The insertion of the acyl chain into hhcyt is assumed to be favored by H-bonding between Asn52 and the protonated phosphate group of CL (Fig. 2) (21).

An alternative model asserts that the binding of CL to hhcyt occurs in the region of the Met80-containing loop, and that the acyl chain protrudes into the protein between the hydrophobic strands formed by residues 67–71 and 82–85 after anchoring of the phospholipid to the protein via electrostatic interactions between the deprotonated phosphate group of CL and the Lys72 residue of hhcyt (Fig. 2) (18).

Very recently, it has been hypothesized that CL binds to hhcyt at two distinct regions (20), which are characterized by different affinity for phospholipids (14, 19). According to this model, the acyl chains of CL could be easily accommodated both in the hydrophobic channel in the vicinity of Asn52 and in the region of the Met80-containing loop (Fig. 2). In the latter case, the deprotonated phosphate group of CL could electrostatically interact with the Lys72 and Lys73 residues. The view that two acyl chains bind to hhcyt at distinct sites is in agreement with the observed two-state transition binding process and with the biphasic character of CL-binding kinetics. This hypothesis is very realistic from a stereochemical viewpoint, in that the insertion of only one acyl chain into hhcyt would cause the
partial exposure to the solvent of (at least) one of the adjacent acyl chains in CL, the situation disfavored from a solvation energy viewpoint (Fig. 2) (20).

As a whole, CL binding to hhcyt seems to induce a gross conformational change(s) on both the proximal and distal side of the heme, causing the loss of the electron transfer properties of hhcyt and its transformation into a globin- and peroxidase-like heme-protein (15, 17, 27–29).

THE CL-BOUND HORSE HEART CYTOCHROME c DISPLAYS PROAPOPTOTIC AND ANTI-APOPTOTIC PROPERTIES

The CL-hhcyt interaction plays an important role in modulating the heme-protein functions. CL-bound cyt shows a non-native tertiary structure and a disrupted heme-Fe-Met80 distal bond, with Lys79 as the likely sixth axial ligand of the heme-Fe-atom at pH > 9 (14, 18–22). CL binding to hhcyt induces a drastically reduced midpoint potential, which falls out of the range required for its physiological role in mitochondrial respiration (17). Further, the cleavage of the distal Fe-Met80 bond endows hhcyt with proapoptotic activity, due to the achievement of peroxidase action (15, 23–26, 31, 32), and anti-apoptotic functions, increasing the affinity for CO and NO (27, 28) and inducing peroxynitrite detoxification properties (29).

Proapoptotic Activity of CL-bound Horse Heart Cytochrome c

At the first stage of apoptosis, cyt released into the cytosol binds to apoptosis-protease activating factor-1. This event starts the process leading to the cleavage of protein substrates and subsequent cell death. Hhcyt release is induced by the dissociation of the CL-hhcyt complex; hhcyt-mediated CL peroxidation is indicated as the process responsible for such an event, although the exact mechanism governing it is not yet fully understood. CL peroxidation is responsible for hhcyt detachment from the mitochondrial membrane. This supported by several evidences, such as the observation that the oxidative degradation of CL occurs in the p53-induced apoptosis (33) and that CL hydroperoxides show a decreased affinity for hhcyt with respect to CL (4, 31). CL oxidation leads to structural changes of hhcyt, such as partial protein unfolding, weak axial binding to the heme iron, and enhanced access of the heme catalytic site to small molecules (e.g., hydrogen peroxide), which result in a modification of the hhcyt catalytic properties, transforming it into a peroxidase (8, 34).

Antiapoptotic Activity of CL-bound Horse Heart Cytochrome c

CL binding to hhcyt facilitates CO and NO binding to the heme-Fe-atomin and peroxynitrite isomerization (27–29). While native ferrous hhcyt (hhcyt-Fe(II)) is unable to bind CO (35), ferrous CL-hhcyt (CL-hhcyt-Fe(II)), like mammalian myoglobin (Mbs) (36), interacts with CO by a simple second-order process (27). The value of the apparent dissociation equilibrium constant for CL-hhcyt-Fe(II) carbonylation (~3 × 10^{-8} M) (27) corresponds to that reported for ferrous horse heart Mb (Mb-Fe(II)) generally taken as a molecular model (~3 × 10^{-8} M) (36). However, both combination and dissociation rate
The unusual features of CO and NO binding to the distal and proximal sides of the heme-Fe-atom, respectively, may represent a new mechanism in the regulation of biological processes by sensing changes in the concentration of CO and NO. Remarkably, a similar situation has been reported for gas discrimination by cytochromes c, a distinct family of class IIa cytochromes found in the periplasm of certain denitrifying, nitrogen-fixing, photosynthetic, methanotrophic, and sulfur-oxidizing bacteria (40).

As reported for pentacoordinated ferric heme-proteins, including horse heart and sperm whale Mb (41–43), ferric Cl-hhcyt (CL-hhcyt-Fe(III)) quickly catalyzes peroxynitrite scavenging, inducing the formation of nitrate (the process takes 10−3 to 1 s) (29). The value of the second-order rate constant for CL-hhcyt-Fe(III)-mediated scavenging of peroxynitrite (3.2 × 105 M−1 s−1) (29) is similar to those reported for ferric heme-proteins (ranging between 1.2 × 104 M−1 s−1 and 4.1 × 103 M−1 s−1) (41–43). However, it has also been shown that peroxynitrite very slowly induces the nitration of the solvent-exposed Tyr74 residue of hhcyt in the absence of CL (the process takes 10 to 30 min). This leads to the cleavage of the Fe-Met80 bond, which is substituted by a weak Fe-Lys72 heme distal ligation (44).

Kinetic and thermodynamic parameters for CO and NO binding to CL-hhcyt-Fe(II) are very favorable, suggesting that the physiological levels of CO and NO may impair CL-hhcyt pro-apoptotic action inhibiting the cyt c peroxidase activity (27, 28). Moreover, peroxynitrite scavenging by CL-hhcyt-Fe(III) may protect the mitochondrion from reactive nitrogen and oxygen species helping cell survival (29).

CONCLUSION AND PERSPECTIVES

CL binding to hhcyt induces tertiary changes facilitating the cleavage of the distal heme-Fe-Met80 bond, and this favors the enzymatic activity of CL-hhcyt (40). Data reported so far indicate that CL-hhcyt could act as either a proapoptotic or an antiapoptotic factor, depending on the conditions under which it operates. In particular, CL-hhcyt functions as a proapoptotic factor catalyzing the peroxidative reduction of H2O2, which leads to CL peroxidation (31, 34). In contrast, CL-hhcyt exerts an antiapoptotic action facilitating the isomerization of peroxynitrite to nitrate with the consequent scavenging of reactive nitrogen species (29). Further, the reaction of CO and NO with CL-hhcyt-Fe impairs the hhcyt peroxidase activity (27, 28).

As a whole, CL-cyt c could act as a modulator of the apoptotic cascade depending on the levels and the type of oxidizing species present in the cellular microenvironment. This pathway appears more complicated in that cytosolic cyt c would bind to several (macro)molecules. Note that cyt c could interact with neuroglobin, a recently discovered neuroprotectant globin in neurons (45–49). The neuroglobin-cyt c interaction would be significantly enhanced by the electrostatic interactions between the two proteins, as cyt c is an unusually basic protein (pI = 4.1).
10.2) while neuroglobin is an acidic protein (pI = 4.6). Thus, at neutral pH, neuroglobin would be highly negatively charged, whereas cyt c would be highly positively charged. The finding that ferrous neuroglobin and ferric cyt c could react leads to the hypothesis that neuroglobin can inhibit cyt c apoptotic activity allowing cell survival under stress conditions by reducing the heme-Fe-atom of cyt c (48, 49). Moreover, several cytosolic and mitochondrial tRNAs specifically associate with cyt c (50). Notably, tRNA blunted the ability of cyt c to induce apoptosis while degradation of tRNA by a RNase enhanced apoptosis via the intrinsic pathway. The cyt-c-tRNA interaction (a previously unexpected connection between two ancient molecules) is pivotal in apoptosis, and may represent an evolutionarily conserved connection between metabolism and cell survival (50, 51).

As a whole, the ability of triggering either proapoptotic and antiapoptotic processes renders cyt c complexes with (macro)molecules crucial elements for the regulation of the cell fate, thus

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