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Review

Apoptotic interactions of cytochrome *c*: Redox flirting with anionic phospholipids within and outside of mitochondria

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Abstract

Since the (re)discovery of cytochrome *c* (cyt *c*) in the early 1920s and subsequent detailed characterization of its structure and function in mitochondrial electron transport, it took over 70 years to realize that cyt *c* plays a different, not less universal role in programmed cell death, apoptosis, by interacting with several proteins and forming apoptosomes. Recently, two additional essential functions of cyt *c* in apoptosis have been discovered that are carried out via its interactions with anionic phospholipids: a mitochondria specific phospholipid, cardiolipin (CL), and plasma membrane phosphatidylserine (PS). Execution of apoptotic program in cells is accompanied by substantial and early mitochondrial production of reactive oxygen species (ROS). Because antioxidant enhancements protect cells against apoptosis, ROS production was viewed not as a meaningless side effect of mitochondrial disintegration but rather playing some – as yet unidentified – role in apoptosis. This conundrum has been resolved by establishing that mitochondria contain a pool of cyt *c*, which interacts with CL and acts as a CL oxygenase. The oxygenase is activated during apoptosis, utilizes generated ROS and causes selective oxidation of CL. The oxidized CL is required for the release of pro-apoptotic factors from mitochondria into the cytosol. This redox mechanism of cyt *c* is realized earlier than its other well-recognized functions in the formation of apoptosomes and caspase activation. In the cytosol, released cyt *c* interacts with another anionic phospholipid, PS, and catalyzes its oxidation in a similar oxygenase reaction. Peroxidized PS facilitates its externalization essential for the recognition and clearance of apoptotic cells by macrophages. Redox catalysis of plasma membrane PS oxidation constitutes an important redox-dependent function of cyt *c* in apoptosis and phagocytosis. Thus, cyt *c* acts as an anionic phospholipid specific oxygenase activated and required for the execution of essential stages of apoptosis. This review is focused on newly discovered redox mechanisms of complexes of cyt *c* with anionic phospholipids and their role in apoptotic pathways in health and disease.

Keywords: Cytochrome *c*; Phospholipid; Mitochondrion

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"Happy families are all alike; Every unhappy family is unhappy in its own way." Lev Tolstoy, "Anna Karenina"

As discerning as the above principle postulated by Lev Tolstoy is in describing societal interactions, it is entirely inapplicable on a cellular level. In fact, quite the opposite can be said about cells: all happy cells are happy in their own well differentiated ways, while unhappy, injured cells are all alike in the ways they end their life through two major death pathways — necrosis or apoptosis. Decoding these pathways, particularly apoptosis has become one of the important foci of cell biology research and new important details are incessantly emerging. This review concentrates on a new role that cytochrome *c* (cyt *c*) complexes with anionic phospholipids – a mitochondria-specific phospholipid, cardiolipin (CL), and plasma membrane phosphatidylserine (PS) – play in apoptotic signaling. We will consider newly discovered pathways through which complexes of cyt *c* with CL and PS, respectively act as a mitochondrial "sensor" during the execution phase of apoptosis and/or as an externalized plasma membrane "eat-me" signal during apoptotic corpse removal (programmed cell clearance). Some of the implications for these lipid-dependent signaling events in human disease will also be discussed.

1. Electron donor/acceptor functions of cytochrome *c* in mitochondria

Cyt *c* is an abundant hemoprotein whose concentration in the intermembrane space of mitochondria may be as high as 0.5–1.0 mM [1]. This renders cyt *c* an effective shuttle of electrons between respiratory complexes III and IV, a function essential for uninterrupted energy metabolism. The quantitatively significant presence of cyt *c* at the mitochondrial membrane crossroads also makes it a potentially important participant of other redox reactions in which not only its electron acceptor/donor features but its catalytic properties are essential. Both participation in electron transport and action as an oxidant of superoxide radicals (a newly ascribed antioxidant function of cyt *c* [2]) are based on tunneling of electrons to its heme-iron and do not require immediate interactions of the heme with the electron donors, complex III or superoxide anion radicals, respectively.

Hexa-coordinate arrangement of cyt *c* heme iron whereby tetra-coordinate association with porphyrin and two additional coordinate bonds with Met_{80} and His_{18}, respectively, ideally fit these electron donor/acceptor functions. In fact, the hexa-coordinate structure precludes involvement of cyt *c* in other duties, such as redox-catalysis of peroxidase reactions, typical of many hemoproteins [3,4]. In line with this, peroxidase activity of solubilized cyt *c* is very low [5]. Interestingly, Met_{80} can undergo oxidative modifications resulting in loss of cyt *c*'s hexa-coordinate state [6,7]. Not surprisingly, oxidants – H_2O_2, organic hydroperoxides – can convert cyt *c* into a peroxidase via oxidation of its Met_{80} [8–10]. However, the physiological relevance of these harsh oxidative conditions resulting in modified forms of cyt *c* with pronounced peroxidase activity remained uncertain. It has been known for a long time that different negatively charged membrane-active molecules such as detergents and some phospholipids can bind to cyt *c* in model systems and stimulate its peroxidase activity [11,12]. The importance of cyt *c* conversion into a peroxidase in vivo is not fully understood.

2. Cardiolipin binding confers peroxidase activity on cytochrome *c*

Recently, we have reported that CL, which is essential for normal functions of several mitochondrial protein complexes [13–16], avidly binds to cyt *c* resulting in an extraordinary enhancement of its catalytic peroxidase activity [17]. The binding includes initial electrostatic attractions of positively charged Lys residues (likely 72 and 73) with negatively charged phosphate groups on CL followed by hydrophobic interactions of one of the polyunsaturated fatty acid residues of CL with a hydrophobic pocket of cyt *c* [18]. Tight interaction between cyt *c* and CL likely involves formation of the hydrogen bond between CL and Asn_{52} in cyt *c* [18]. The binding constants of cyt *c* with different polyunsaturated molecular species of CL are very high (on the order of 10^9 M^{-1}); thus the complexes produced cannot be easily dissociated by disruptors of electrostatic interactions such as high ionic strength conditions [147].

Cyt *c* has a highly conserved primary structure across different species. As shown in Fig. 1, a particular segment of the cyt *c* molecule that includes Met_{80} as well Lys_{72}, Lys_{73} and Tyr_{48}, Tyr_{67}, Tyr_{74} remains invariant in different species. Because these particular amino acid residues are essential for either binding of cyt *c* with CL (Lys_{72}, Lys_{73}) or likely participate in realization of its catalytic peroxidase activity (Met_{80}, Tyr_{48}, Tyr_{67}, Tyr_{74}), it is tempting to speculate that this peroxidase function and structural organization of cyt *c* once emerged, remained evolutionary conserved.

In normal mitochondria, CL is confined to the inner (about 65% of total CL) and outer (about 35% of total CL) leaflets of the inner mitochondrial membrane (IMM) whereas its presence in the outer mitochondrial membrane (OMM) is negligible [17,19,20]. Thus, physical contact between cyt *c* and CL can only take place on the intermembrane surface of the outer IMM leaflet. Furthermore, a significant fraction of CL is localized within the contact sites between the IMM and OMM [21–23] and is also engaged in interactions with other mitochondrial proteins [13–16,24]. As a result, only a limited amount of CL is available for binding with cyt *c*. Consequently, cyt *c*/CL complexes normally represent only a small portion of both cyt *c* (about 10–15%) and CL (2–3%) [17,25].

Recent focus on the involvement of CL in apoptosis revealed that the triggering mechanisms engaging Bcl-2 family members require CL [22,23,26,27] and cause massive trans-membrane migration of CL as well as its hydrolysis [28,29]. Both peroxidation and hydrolysis of CL occur during apoptosis and are important in its execution, particular in release of pro-apoptotic factors [17,30]. One of the members of the Bcl-2 family of proteins, Bid (more specifically, truncated Bid or tBid), appears early in apoptotic