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Review

Functional role of cardiolipin in mitochondrial bioenergetics ☆

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ABSTRACT

Cardiolipin is a unique phospholipid which is almost exclusively located in the inner mitochondrial membrane where it is biosynthesized. Considerable progress has recently been made in understanding the role of cardiolipin in mitochondrial function and bioenergetics. This phospholipid is associated with membranes designed to generate an electrochemical gradient that is used to produce ATP, such as bacterial plasma membranes and inner mitochondrial membrane. This ubiquitous and intimate association between cardiolipin and energy transducing membranes indicates an important role for cardiolipin in mitochondrial bioenergetic processes. Cardiolipin has been shown to interact with a number of proteins, including the respiratory chain complexes and substrate carrier proteins. Over the past decade, the significance of cardiolipin in the organization of components of the electron transport chain into higher order assemblies, termed respiratory supercomplexes, has been established. Moreover, cardiolipin is involved in different stages of the mitochondrial apoptotic process, as well as in mitochondrial membrane stability and dynamics. This review discusses the current understanding of the functional role that cardiolipin plays in several reactions and processes involved in mitochondrial bioenergetics. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

Cardiolipin (CL) is commonly referred to as the signature phospholipid of the mitochondria. Among phospholipid species, CL has interesting chemical and structural characteristics, being highly acid and having a head group (glycerol) that is esterified to two phosphatidylglyceride backbone fragments rather than one, resulting in a very specific ultrastructure and role in the mitochondrial function. The diphosphatidylglycerol structure combined with four acyl chains gives cardiolipin its dimeric nature, which is unique among phospholipids and results in high specific conical structure. Cardiolipin is almost exclusively associated with membranes designed to produce ATP through the electrochemical gradient generated by the electron transport chain. Such membranes include the bacterial plasma membrane [1] and the inner mitochondrial membrane [2,3]. This ubiquitous and intimate association between CL and energy-transducing membranes suggests an important role for CL in mitochondrial bioenergetic processes.

Cardiolipin has been shown to interact with a number of inner mitochondrial membrane (IMM) proteins, enzymes and metabolite carriers [4–6]. The list of proteins that bind cardiolipin with high affinity is long and includes, among others, the electron transport chain complexes involved in oxidative phosphorylation (OXPHOS) and ADP/ATP carrier (AAC) (Fig. 1). Indeed, CL is required for optimal activity of complex I (NADH–ubiquinone oxidoreductase) [7–9], complex III (ubiquinone–cytochrome c oxidoreductase) [7,10,11], complex IV (cytochrome c oxidase) [12], and complex V (ATP synthase) [13]. Crystallographic studies have shown the presence of a few tightly bound CL molecules in each of the crystal structures of complex III [11], complex IV [14], and ADP/ATP carrier [15] as well as in crystallized prokaryotic proteins, such as the photoreaction center [16], the trimeric formate dehydrogenase-N [17] and succinate dehydrogenase [18]. These results suggest that CL is an integral component of these proteins, the presence of which is critical to folding.

Mitochondrial respiratory chain complexes assemble in higher order structure referred to generically as respiratory supercomplexes [19–21]. The unique, dimerically cross-linked phospholipid structure of CL seems to affect the stability and activity of such respiratory supercomplexes. Indeed, respiratory supercomplexes consisting of complexes III and IV are destabilized in mitochondria lacking CL [22–24]. Similarly, dimers of ADP/ATP carrier and other ADP/ATP carrier-containing complexes dissociated in CL-deficient mitochondria [22,25]. These examples illustrate the important role of CL for mitochondrial bioenergetic function; but in addition, recent studies are now revealing that CL has a much broader impact on mitochondrial physiology and pathophysiology [4–6].

Cardiolipin has been implicated in the process of apoptosis in animal cells through its interaction with a variety of death-inducing proteins, including cytochrome c (Cyt c) [26–29]. Cardiolipin-bound Cyt c acts

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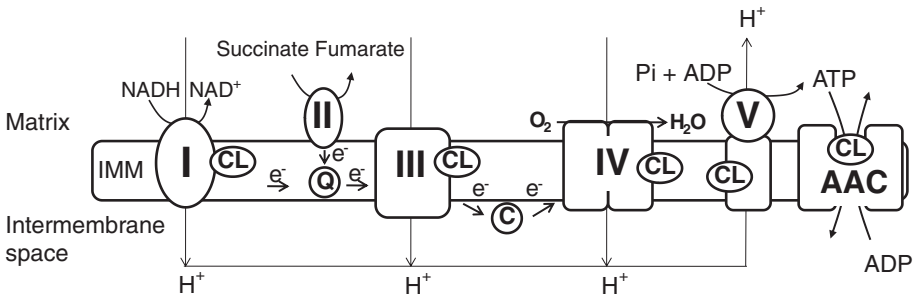

Fig. 1. Interaction of CL with oxidative phosphorylation complexes. The electrons are transferred along the path shown in the figure, resulting in the reduction of oxygen to water at complex IV. During this process, protons (H^+) are pumped by complexes I, III and IV into the intermembrane space to form an electrochemical gradient which is utilized by complex V to synthesize ATP from ADP and inorganic phosphate (Pi). ATP formed is then transferred by the ADP/ATP carrier (AAC) to the intermembrane space in exchange with ADP. Cardiolipin binds to complexes I, III, IV, and V and AAC. Q, coenzyme Q; C, cytochrome c; CL, cardiolipin.

as a peroxidase capable of catalyzing H_2O_2-dependent CL peroxidation, which is an essential step in the release of Cyt c during apoptosis [30]. Another function for CL in relation to energy metabolism is that it anchors two kinases, mitochondrial creatine kinase and nucleoside diphosphate kinase to the inner and possibly to outer mitochondrial membrane (OMM), where they come in contact [31,32].

Due to the role played by CL in mitochondrial bioenergetics as well as in apoptosis, it is conceivable that CL abnormalities may have important implications in mitochondrial dysfunction and hence, in cellular pathophysiology. Alterations in CL structure, content and acyl chain composition, associated with mitochondrial dysfunction, have been described in several pathophysiological conditions, such as hypo–hyperthyroid states [33–37], heart ischemia–reperfusion [38–42], nonalcoholic fatty liver disease [43], diabetes [44,45], Barth syndrome [46,47] and aging [48–51].

In this review, we discuss the current state of knowledge of the role played by CL in several reactions and processes involved in mitochondrial bioenergetics.

2. Cardiolipin and mitochondrial substrate carriers

The primary function of mitochondria is the synthesis of ATP by oxidative phosphorylation. In addition to this important role, these organelles are also the locus of other essential metabolic pathways, such as the citric acid cycle, fatty acid oxidation, the synthesis and degradation of amino acids (urea cycle), and the synthesis of iron–sulfur clusters and heme. To carry out these pathways, metabolites have to be continuously exchanged between the mitochondrial matrix and the cytoplasm. The inner membrane contains a mitochondrial carrier family (MCF) that catalyzes the transport of a number of metabolites between the intramembrane and matrix space [52]. Defined human diseases are now known to result from mutations in several members of this family [52]. One defining feature of the carriers is the so-called tripartite structure, consisting of three homologous sequence repeats of about 100 amino acid residues each, which was first noted in the sequence of the bovine ADP/ATP carrier [15].

Mitochondrial solute carriers constitute a major part of the inner membrane proteins. Several studies have shown that CL interacts with a number of mitochondrial carrier proteins and it is required for their optimal activity [53]. The mitochondrial phosphate carrier requires CL for its activity in proteoliposomes, and other phospholipids cannot substitute for CL [54]. In the heart, oxidation of pyruvate and β-oxidation of fatty acids are two major sources of ATP generation. The transport of pyruvate into the mitochondria by the pyruvate carrier and the exchange of carnitine esters by the carnitine: acylcarnitine translocase are critical for energy metabolism. The activities of both these translocases, reconstituted in proteoliposomes, have been shown to be most efficient in the presence of CL, and this could not be achieved by other phospholipids [55,56]. The tricarboxylate carrier has been demonstrated to be stimulated in the presence of CL, although the same stimulatory effect is observed in the presence of other phospholipids, such as phosphatidylserine and phosphatidylinositol [52]. Facing membrane lipids consisting of up to 20% CL, it is not surprising that mitochondrial carrier proteins interact with CL, this phospholipid being one of the major lipid components of the IMM. On solubilization of mitochondrial carriers, detergents replace to a large extent the membrane lipids. Without their native environment, the carriers become labile and the supplementation of phospholipids, especially of cardiolipin, may protect and facilitate the purification of the carrier in a functionally intact state. Preservation and stabilization of the native state of these carrier proteins by CL could explain the increased transport activity of these proteins, when measured in the reconstituted proteoliposomes. The importance of CL in maintaining the full catalytic activity of these carrier proteins may result from its unique large head group carrying two negative charges, requiring a specific and tightly interacting binding site, which may stabilize a protein domain in a clamp-like manner.

The ADP/ATP carrier plays an important role in energy metabolism by allowing the ATP formed by oxidative phosphorylation to pass across the IMM to intermembrane space. The movement of ATP is coupled by an antiport mechanism resulting in the 1:1 exchange of ATP for external ADP. Interestingly, the activity of the AAC has been shown to be optimal only in the presence of tetralinoleoyl–CL [53]. Other CL species, such as tetraoleoyl–CL and monolyso–CL, and also other phospholipids, were not effective in stimulating the AAC activity. There is also evidence from studies in yeast that absence of CL destabilizes the interaction of ACC with other mitochondrial proteins [25].

ADP/ATP carrier is the most abundant protein in the IMM (up to 10% of membrane proteins in bovine heart mitochondria), and this allowed for a detailed investigation of the carrier–CL interaction at molecular level. In fact, the availability of large amount of purified ACC protein and the strong stabilization of the structure by inhibitor ligand carboxyactractyloside (CAT), thus preventing protein unfolding and degradation [57], have allowed for the purification of the protein and its crystallization in the CAT-bound form. The crystal structure demonstrated that there are either two molecules of CL bound per monomer of ACC [15], or three molecules of CL bound per monomer of ACC [58]. The additional tightly bound CL found in the second crystal structure seems to be important in stabilizing protein–protein interactions in the crystal [15]. In addition to this X-ray structures, parallel ^{31}P NMR measurements have revealed the presence of tight bound CL to AAC [59]. The tight binding of CL can be rationalized in view of the large excess of positive charges in the AAC. A number of lysine residues have been suggested to face the lipid bilayer at the level of the membrane surface [53]. It was proposed that the CL molecules are bound at the lysine residues such that their headgroups face the matrix side. CL has the negative charges distributed over a large headgroup having two phosphates separated by glycerol. It is possible that the head group structure of CL allows it to bind optimally to sites on ACC. From the crystal structure

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