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Evaluation of the cardiolipin biosynthetic pathway and its interactions in the diabetic heart

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ABSTRACT

Aims: We have previously reported alterations in cardiolipin content and inner mitochondrial membrane (IMM) proteomic make-up specifically in interfibrillar mitochondria (IFM) in the type 1 diabetic heart; however, the mechanism underlying this alteration is unknown. The goal of this study was to determine how the cardiolipin biosynthetic pathway and cardiolipin–IMM protein interactions are impacted by type 1 diabetes mellitus.
Main methods: Male FVB mice were made diabetic by multiple low-dose streptozotocin injections and sacrificed five weeks post-diabetic onset. Messenger RNA was measured and cardiac mitochondrial subpopulations were isolated. Further mitochondrial functional experimentation included evaluating the protein expression of the enzymes directly responsible for cardiolipin biosynthesis, as well as ATP synthase activity. Interactions between cardiolipin and ATP synthase subunits were also examined.
Key findings: Western blot analysis revealed a significant decrease in cardiolipin synthase (CRLS) protein content in diabetic IFM, with a concomitant decrease in its activity. ATP synthase activity was also significantly decreased. We identified two novel direct interactions between two subunits of the ATP synthase F_0 complex (ATP5F1 and ATP5H), both of which were significantly decreased in diabetic IFM.
Significance: Overall, these results indicate that type 1 diabetes mellitus negatively impacts the cardiolipin biosynthetic pathway specifically at CRLS, contributing to decreased cardiolipin content and loss of interactions with key ATP synthase F_0 complex constituents in the IFM.

Introduction

Cardiovascular complications, including diabetic cardiomyopathy are the primary cause of morbidity in diabetic patients. Diabetic cardiomyopathy has been associated with mitochondrial dysfunction (Devereux et al., 2000; Severson, 2004; Tomita et al., 1996). The inner mitochondria membrane (IMM) houses vital processes in a specific lipid environment. Alteration in the IMM lipid environment has been associated with dysfunction to mitochondrial processes (Acehan et al., 2011a; Chicco and Sparagna, 2007; Han et al., 2005; Jiang et al., 2000). Proteomic analyses of type 1 diabetic mitochondria indicates that IMM proteins may be particularly prone to damage and loss (Baseler et al., 2011).

Impaired mitochondrial function, particularly in those processes situated in the IMM, may be associated with disruption to the phospholipid environment contained within. Most abundant in the mammalian heart, cardiolipin is a unique phospholipid, primarily located in the IMM (Schlame et al., 2000) and is thought to play a critical role in mitochondrial structure and bioenergetics (Acehan et al., 2011a). The biosynthetic pathway (Fig. 1) begins in the outer mitochondrial membrane where phosphatidic acid is converted into cytidine diphosphate diacylglycerol (CDP-DAG) by cytidine diphosphate diacylglycerol synthase (CDS) (Houtkooper and Vaz, 2008). Phosphatidylglycerol synthase (PGS) then converts CDP-DAG to phosphatidylglycerol phosphate (PG-P), where the phosphate is removed by phosphatidylglycerol phosphatase (PGP), resulting in the formation of phosphatidylglycerol (PG). Cardiolipin synthase (CRLS) then catalyzes the condensation of CDP-DAG and PG, resulting in a nascent form of cardiolipin (Chicco and Sparagna, 2007; Lu et al., 2006). Because the nascent cardiolipin does not contain the precise fatty acyl side chains for the specific tissue, a remodeling process takes place, where tafazzin (TAZ) or monolysocardiolipin acyltransferase, converts the nascent cardiolipin to mature cardiolipin containing specific fatty acyl side chains (Chicco and Sparagna, 2007), which is dependent upon the tissue and environment. Linoleic acid, the most abundant fatty acid found in cardiac mitochondria, constitutes approximately 80% of all side chains (Acehan et al., 2011b; Schlame et al., 2000). The properties of cardiolipin allow it to interact with IMM proteins and facilitate proper mitochondrial function.

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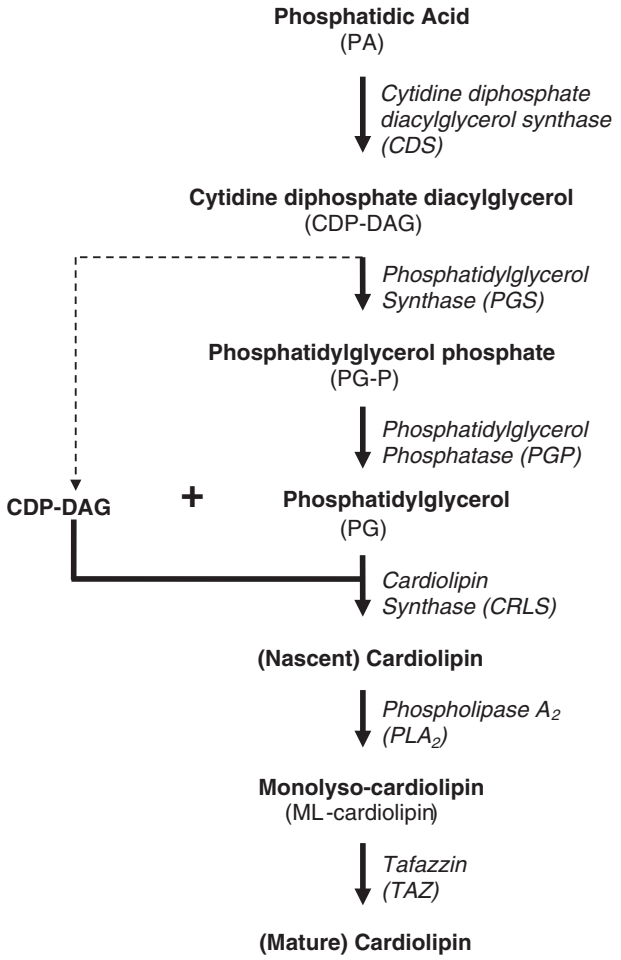

Fig. 1. Cardiolipin biosynthetic pathway. Schematic diagram illustrating cardiolipin biosynthetic pathway.

Literature suggests an interaction between cardiolipin and the electron transport chain (ETC) complexes involved in oxidative phosphorylation (Schlame et al., 2000), including complexes I, III, IV, and ATP synthase (Fry and Green, 1981; Sedlak and Robinson, 1999). Cardiolipin plays a critical role in the organization of mammalian mitochondrial ATP synthase and it has been suggested that the F_0 complex of ATP synthase contains high affinity binding sites for cardiolipin (Acehan et al., 2011a); however, the specific binding sites remain undefined. Cardiolipin also serves as a proton reservoir for maintenance of IMM potential thus, contributing to ATP synthesis (Haines and Dencher, 2002). Shotgun lipidomics revealed decreased tetralinoleic cardiolipin content in diabetic hearts suggesting a possible mechanistic reliance on the interaction between IMM protein activity and cardiolipin (Han et al., 2005). We have previously reported decreased ETC protein content and function in IFM following a type 1 diabetic insult (Baseler et al., 2011;Dabkowski et al., 2009), correlating with decreased tetralinoleic cardiolipin content, with no effect on subsarcolemmal mitochondria (SSM) situated beneath the plasma membrane (Dabkowski et al., 2009).

To date, the impact of type 1 diabetes mellitus on the cardiolipin biosynthetic pathway in mitochondrial subpopulations and on the interactions between cardiolipin and IMM proteins has not been studied. We hypothesized that the decreased cardiolipin content associated with type 1 diabetes mellitus results from defective cardiolipin biosynthesis influencing its association with IMM proteins, with the effects being most pronounced in IFM.

Materials and methods

Experimental animals and induction of diabetes

The animal experiments in this study conformed to the National Institutes of Health (NIH) *Guidelines for the Care and Use of Laboratory Animals* and were approved by the West Virginia University Animal Care and Use Committee. Male FVB mice were housed in the West Virginia University Health Sciences Center animal facility on a 12-h light/dark cycle in a temperature controlled room. Mice were given unlimited access to a standard rodent diet and water. Type 1 diabetes mellitus was induced in 6-week-old mice following the protocol of the Animal Models of Diabetic Complications Consortium using multiple low-dose streptozotocin (STZ; Sigma-Aldrich Corporation, St. Louis, MO) intraperitoneal injections. The STZ-induced type 1 diabetic model was chosen because it is the most widely utilized model and the multiple low dose administration is not associated with many of the deleterious side effects observed with the single high-dose approach (Wu and Huan, 2001). Injections of 50 mg/kg body weight STZ dissolved in sodium citrate buffer (pH 4.5) were performed daily for 5 consecutive days after 6 h of fasting. Mice serving as vehicle controls were given the same volume per body weight of sodium citrate buffer. One week post-injection, hyperglycemia was confirmed by measuring blood glucose (Contour Blood Glucose test strips; Bayer, Mishawaka, IN), where >250 mg/dL was considered diabetic. Five weeks after the onset of hyperglycemia, animals were sacrificed for experimentation. Blood glucose levels were again tested at this time and all remained >250 mg/dL.

Mitochondrial subpopulation isolation

Five weeks post-diabetic onset, FVB mice and their littermate controls were sacrificed and hearts excised. Hearts were rinsed in phosphate buffered saline (PBS, pH 7.4), then blotted dry. SSM and IFM were isolated as previously described by Palmer et al. (1977) with modifications by our laboratory (Dabkowski et al., 2008, 2009; Williamson et al., 2010). Mitochondrial pellets were resuspended in either KME buffer (100 mM KCl, 50 mM MOPS, and 0.5 mM EDTA) for Western blot analysis and activity measurements, or in 1.0% NP-40 wash/binding buffer (10 mM HEPES (pH 7.4), 1.0% NP-40, 150 mM NaCl) for cardiolipin/protein interactions. Protein content was determined by the Bradford method using bovine serum albumin as a standard (Bradford, 1976).

Cardiolipin analyses

Isolated mitochondrial subpopulations from control and diabetic hearts were pooled, and sent off to Avanti Polar Lipids, Inc. (Alabaster, AL) for cardiolipin analysis. Extraction and analysis of cardiolipin species were based on the protocols of Sparagna et al. (2005) and Minkler and Hoppel (2010). In brief, cardiolipin detection was performed by liquid chromatography (Dionex U-3000 binary HPLC)/hybrid tandem mass spectrometry using a quadrapole-linear ion trap (API 4000 Qtrap) operated in the negative ion mode. Mass spectrometer instrument conditions included a spray voltage of −4.5 kV, capillary voltage of −150 V, heated capillary temperature of 400 °C, and a sheath gas flow rate of 10 arbitrary units. Mitochondrial cardiolipin and internal standard spectra were identified by multiple reaction monitoring. All experiments were performed in triplicate.

mRNA isolation and analysis

mRNA levels were examined as previously described (Baseler et al., 2011). Briefly, hearts were rinsed in phosphate buffered saline (PBS, pH 7.4) then snap frozen in liquid nitrogen. Thirty milligrams of tissue was excised from control and diabetic hearts from which the mRNA was extracted using an RNeasy mini kit, per manufacturer's instructions