

Regulation of cardiolipin biosynthesis in the heart

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Abstract

Cardiolipin is one of the principle phospholipids in the mammalian heart comprising as much as 15–20% of the entire phospholipid phosphorus mass of that organ. Cardiolipin is localized primarily in the mitochondria and appears to be essential for the function of several enzymes of oxidative phosphorylation. Thus, cardiolipin is essential for production of energy for the heart to beat. Cardiac cardiolipin is synthesized via the cytidine-5'-diphosphate-1,2-diacyl-*sn*-glycerol pathway. The properties of the four enzymes of the cytidine-5'-diphosphate-1,2-diacyl-*sn*-glycerol pathway have been characterized in the heart. The rate-limiting step of this pathway is catalyzed by the phosphatidic acid: cytidine-5'-triphosphate cytidyltransferase. Several regulatory mechanisms that govern cardiolipin biosynthesis in the heart have been uncovered. Current evidence suggests that cardiolipin biosynthesis is regulated by the energy status (adenosine-5'-triphosphate and cytidine-5'-triphosphate level) of the heart. Thyroid hormone and unsaturated fatty acids may regulate cardiolipin biosynthesis at the level of three key enzymes of the cytidine-5'-diphosphate-1,2-diacyl-*sn*-glycerol pathway, phosphatidylglycerolphosphate synthase, phosphatidylglycerolphosphate phosphatase and cardiolipin synthase. Newly synthesized phosphatidic acid and phosphatidylglycerol may be preferentially utilized for cardiolipin biosynthesis in the heart. In addition, separate pools of phosphatidylglycerol, including an exogenous (extra-mitochondrial) pool not derived from *de novo* phosphatidylglycerol biosynthesis, may be utilized for cardiac cardiolipin biosynthesis. In several mammalian tissues a significant number of studies on polyglycerophospholipid biosynthesis have been documented, including detailed studies in the lung and liver. However, in spite of the important role of cardiolipin in the maintenance of mitochondrial function and membrane integrity, studies on the control of cardiolipin biosynthesis in the mammalian heart have been largely neglected. The purpose of this review will be to briefly discuss cardiolipin and cardiolipin biosynthesis in some selected model systems and focus primarily on current studies involving the regulation of cardiolipin biosynthesis in the heart. (*Mol Cell Biochem* **159**: 139–148, 1996)

Key words: cardiolipin, heart, phospholipid biosynthesis

Abbreviations: CL – cardiolipin; PG – phosphatidylglycerol; PA – phosphatidic acid; CTP – cytidine-5'-triphosphate; CDP-DG – cytidine-5'-diphosphate-1,2-diacyl-*sn*-glycerol; CMP – cytidine-5'-monophosphate; lysoPG – lysophosphatidylglycerol; monolysoCL – monolysocardiolipin; BMP – bis(monoacylglycerol)phosphate; ATP, adenosine-5'-triphosphate; ADP – adenosine-5'-diphosphate; PGP – phosphatidylglycerol phosphate; DNA – deoxyribonucleic acid; STZ – streptozotocin

Introduction

Nomenclature

Three major classes of polyglycerophospholipids exist: a) bis-(1,2-diacyl-*sn*-glycero-3-phospho)-1',3'-*sn*-glycerol; diphosphatidylglycerol or cardiolipin (CL)¹ (Fig. 1); b) PG,

and c) BMP (for comprehensive review see [1]). All three classes are found in most mammalian tissues but CL is the most abundant. In animals CL was found in highest concentration in cardiac muscle. PG was present at concentrations of about 1% of total phospholipid, except in lung, where it comprised 2–5% of the total phospholipid phosphorus mass and is a major component of pulmonary surfactant (for re-

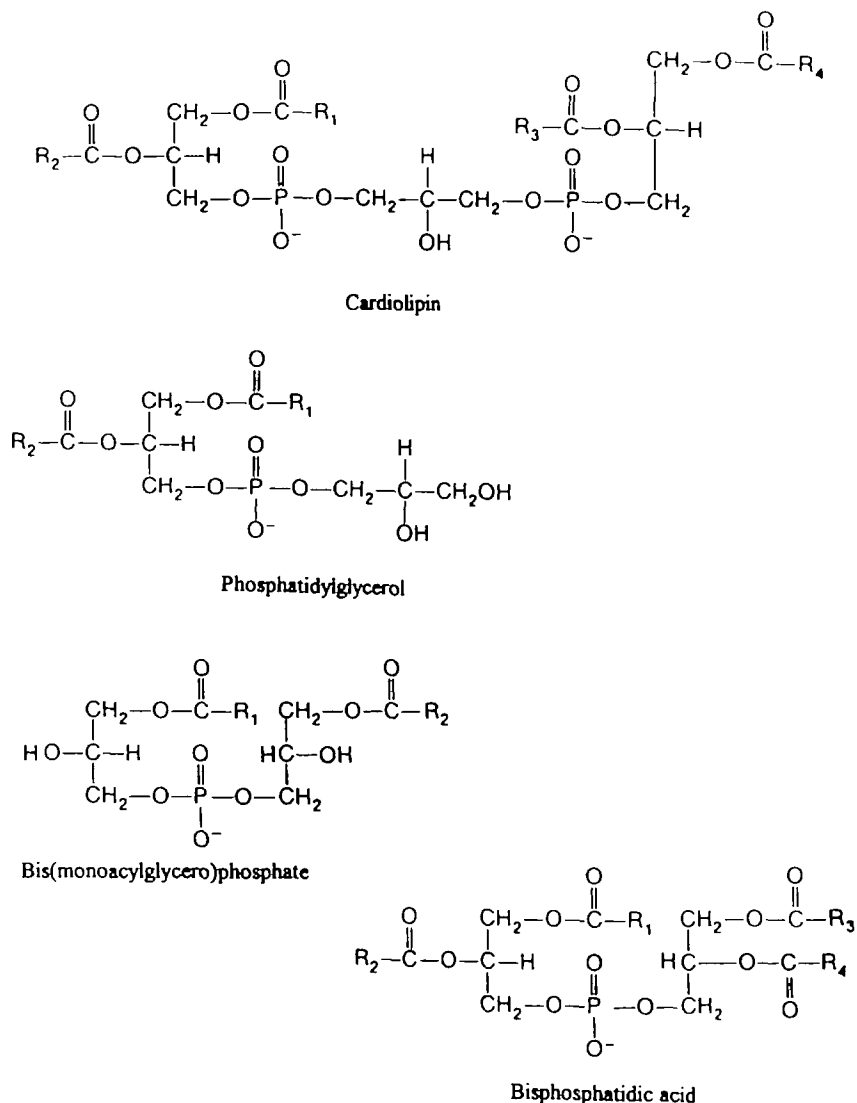


Fig. 1. The structures of the polyglycerophospholipids.

view see [73]). BMP comprised less than 1% of the phospholipid phosphorus mass in normal animal tissue, with the exception of the alveolar macrophage, where it represented as much as 14–18% of total phospholipid [1]. BMP has been implicated as a possible source of arachidonate for eicosanoid biosynthesis [74]. However, BMP was not identified in the heart [2, 3]. Recently, another polyglycerophospholipid, bisphosphatidic acid, was identified in trace amounts in human foreskin fibroblasts and a putative role for this compound in phospholipase D mediated signal attenuation proposed [4]. Treatment of these cells with bradykinin resulted in a phospholipase D-mediated transphosphatidylation from phosphatidylcholine (donor) to endogenous 1,2-diacyl-*sn*-glycerol (acceptor) yielding bisphosphatidic acid. This appeared to be the first demonstration of a physiological role for phospholipase D-mediated transphosphatidylation.

General properties of cardiolipin

CL, the first polyglycerophospholipid discovered, was isolated from beef heart in 1942 by Mary Pangborn [5]. CL has been observed in both eukaryotic and prokaryotic cells [1]. CL was demonstrated to play an important role in DNA replication in *E. Coli* by facilitating the ADP-ATP exchange reaction of dnaA protein [6]. In yeast CL was required for the translocation of proteins across membranes [7]. Antibodies to CL have been associated with several human diseases [8]. However, the biological significance of this is unknown. In the rat heart CL comprised approximately 15% of the total cardiac phospholipid mass [2, 3]. In rat liver, CL was characteristically associated with the inner mitochondrial membrane where it constituted approximately 21% of the total membrane phospholipid mass [9]. Although the majority of CL was localized to the mitochondria, some CL has been ob-

served in microsomes [62]. The potent anthracycline antibiotic adriamycin strongly binds CL and was used as a probe to study the distribution of CL in the inner mitochondrial membrane of rat heart and liver [65, 66]. In both membranes, 57% of the total CL was localized to the cytosolic face of the inner membrane [66]. CL has been shown to be required for the activity of, or intimately associated with, a number of key mitochondrial enzymes including cytochrome c oxidase [10], carnitine palmitoyl-transferase [11], creatine phosphokinase [12], pyruvate translocator [13], tricarboxylate carrier [14], mitochondrial glycerol-3-phosphate dehydrogenase [15] and the phosphate transporter [16, 33]. In Chinese hamster ovary cells the biosynthesis of PG and CL were shown to be essential for cell growth and function of the electron transport chain [21]. The temperature sensitive Chinese hamster ovary mutant, PGS-S, with thermolabile PGP synthase was defective in the biogenesis of both PG and CL at the nonpermissive temperature. This mutant had both morphological and functional mitochondrial abnormalities including impairment of the rotenone-sensitive NADH-ubiquinone reductase (Complex I). When the mutants were placed on a galactose medium, in which 98% of the cellular energy is supplied by oxidative phosphorylation, growth was markedly attenuated compared with wild type cells.

CL's have unique fatty acid molecular species with high levels of polyunsaturated fatty acids, primarily 18:2 (n-6) and low levels of saturated fatty acids [17, 30]. The relative amounts of 18:2 in CL was shown to increase in skeletal muscle during the postnatal period (40%) and reached maximum values (90%) during the first two months after birth [18]. Rats fed a partially hydrogenated marine oil diet, rich in 16:1, 18:1, 20:1 and 22:1 isomeric fatty acids, for 10 weeks exhibited an increase in 18:2 CL molecular species in liver compared to rats fed a palm oil diet [69]. Thus, the fatty acid composition of CL may differ with various diets. The position of the four acyl groups of individual species of various eukaryotic CL were shown to be occupied by similar chains or they differed by two carbon atoms or one double bond, respectively [30]. A microsomal acyltransferase activity was described which catalyzed the transfer of fatty acyl groups from acyl-Coenzyme A to monolysoCL or dilysoCL [70]. More recently, a deacylation-reacylation cycle for remodeling of CL molecular species in rat liver mitochondria was proposed [71]. Newly synthesized CL was rapidly deacylated to monolysoCL and reacylated with linoleoyl-Coenzyme A. DilysoCL was not appreciably reacylated. In addition, pre-existing mitochondrial CL was relatively resistant to this reaction.

Little information is available on the catabolism of cardiac CL. CL was hydrolyzed slowly (or not at all) by most phospholipases C and under some conditions was hydrolyzed by certain of the phospholipases D [1]. In mammalian liver the turnover of CL was much slower than the other phospholipid classes [67]. The endogenous mitochondrial phospholipases A₂ were capable of hydrolyzing CL [1]. In addition,

CL may be degraded in lysosomes to form BMP [72]. The initial attack involved a lysosomal phospholipase A [20]. Formation of monolysoCL was followed by formation of dilysoCL and acyl-di-(glycerophosphoryl)-glycerol. Further lysosomal degradation occurred by one of two pathways; either to di-(glycerophosphoryl)-glycerol or to acylglycerophosphorylglycerol and glycerophosphate then to glycerophosphorylglycerol and glycerol. The latter pathway accounted for greater than 90% of the end products.

Cardiolipin biosynthesis in mammalian tissues

The scheme depicted in Fig. 2 is generally applicable to the biosynthesis of CL in mammalian mitochondria [22, 23]. Biosynthesis of PG in mammalian cells occurs via the CDP-DG pathway first elucidated by Eugene Kennedy and co-workers [22]. CDP-DG is formed from PA, catalyzed by PA:CTP cytidylyltransferase. Subsequently, *sn*-glycerol-3-phosphate and CDP-DG are converted to PG by the sequential action of PGP synthase and PGP phosphatase activities. Some accumulation of PGP has been observed in rat brain incubations [24, 25]. However, in rat heart PGP was rapidly converted to PG and did not accumulate to a significant extent [3]. The PGP synthase and phosphatase have been partially purified from mammalian liver mitochondria [50, 53]. CL is synthesized by a condensation of PG with CDP-DG catalyzed by CL synthase [23]. In liver, the CL synthase was localized to the inner mitochondrial membrane [26, 27]. McMurray and Jarvis [28] solubilized the CL synthase from rat liver mitochondrial membranes using an amphoteric detergent, miranol. The synthesis of CL was dependent on divalent cations and CDP-DG. The CDP-DG requirement was provided by saturated (dipalmitoyl) or unsaturated (dioleoyl) derivatives. Schlame and Hostetler purified the rat liver mitochondrial CL synthase to homogeneity [29]. However, an antibody to this enzyme was never produced due to the small yield of protein. The purified enzyme required cobalt, had an isoelectric point of pH 4-5, a pH optimum of 8-9, and sodium dodecyl gel electrophoresis revealed a molecular weight of 50 kilodaltons. K_m values of 45 and 16 μM for PG and CDP-DG, respectively, were reported. The activity of the purified enzyme was reconstituted by the addition of phospholipids, phosphatidylethanolamine being the most effective. All mitochondrial CL synthases in eukaryotes are inhibited by lysoPG and are insensitive to *n*-ethylmaleimide [30]. None of the other enzymes involved in CL biosynthesis has been purified to homogeneity from any mammalian source. A preliminary report described the biosynthesis of CL via a phospholipase D-mediated transphosphatidyl transfer reaction [75]. Cabbage phospholipase D catalyzed the formation of CL from ³H-labeled phosphatidylglycerol in an ether solution. The biological significance of this reaction is unknown.

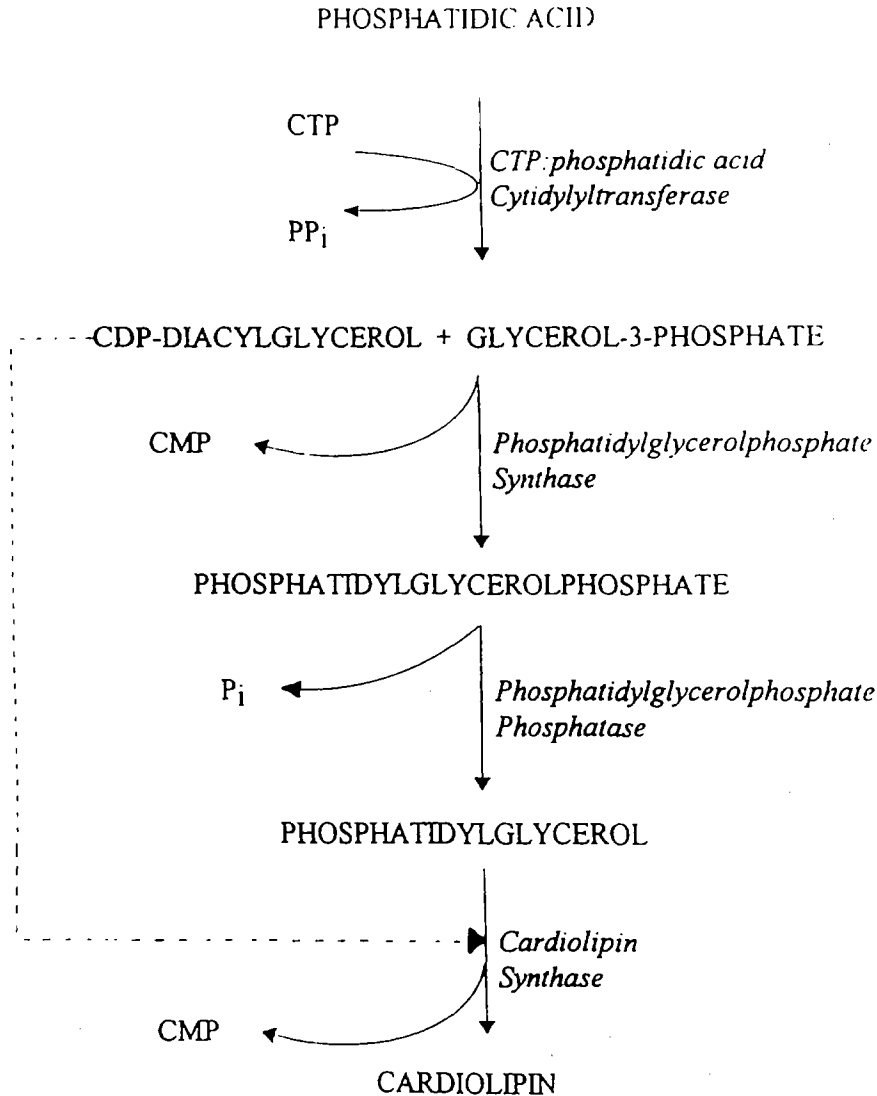


Fig. 2. The CDP-DG pathway of cardiolipin biosynthesis in the heart.

Regulation of cardiolipin biosynthesis in mammalian tissues

Hormones

Rat liver mitochondria contain a thyroid hormone receptor thought to be involved in regulation of mitochondrial oxidative phosphorylation [31]. Treatment of rats with thyroid hormone has been shown to increase liver mitochondrial levels of CL [32]. These alterations in mitochondrial CL levels have been implicated with increased rates of phosphate transport in hyperthyroidism [16]. Mitochondrial levels of CL appear to be regulated in part by the effect of thyroid hormone on the activity of the CL synthase. Treatment of rats with thyroxine for five days increased rat liver mitochondrial CL synthase activity 52% [34]. A 36% decrease in cardiac

CL, observed in hypothyroid rats, was associated with a decrease in the activity of the mitochondrial pyruvate transporter [13], whereas pyruvate transport was stimulated in heart mitochondria of hyperthyroid rats in which the CL content was elevated [35]. In these studies, it was not determined if thyroid hormone treatment affected cardiac CL synthase activity or if cardiac CL biosynthesis was increased. Hypophysectomy of rats resulted in a 25% decrease in liver mitochondrial CL [63]. Injection of bovine growth hormone for seven days restored CL levels to control values. Incubation of cat cardiac muscle slices with [³²P]orthophosphate and glucagon for 30 min did not affect incorporation of [³²P]orthophosphate into CL [64]. In contrast, incorporation of [³²P]orthophosphate into phosphatidylserine was stimulated six-fold in the presence of glucagon.

Concentration of CDP-DG, CTP, CMP

Biosynthesis of mammalian CL is CDP-DG dependent [23]. However, in liver mitochondria the CL synthase appeared to have no selective preference for the CDP-DG molecular species [68]. A potential regulator of CL biosynthesis is the mitochondrial CDP-DG hydrolase [42]. In liver, this hydrolase activity was reported to be several-fold higher than PGP synthase. This hydrolase activity may effectively compete for the small pool of cellular CDP-DG. However, the hydrolase activity was inhibited progressively by divalent cations [42] and these cations stimulated CL synthase activity *in vitro* [68]. Amphiphilic amines (e.g. chlorphentermine, chlorpromazine) are hypotriglyceridemic agents for the metabolic treatment of obesity [36, 37]. These compounds act by decreasing the availability of PA for the conversion to 1,2-diacyl-*sn*-glycerol [38]. The result of which was an enhanced CDP-DG production. In addition, chlorpromazine was shown to inhibit CDP-DG hydrolase activity [39]. When chronically administered, amphiphilic drugs induced an increase in phospholipid content in most tissues [40]. There appeared to be specific accumulation of acidic phospholipids including CL in lysosomes [41]. Thus, the cellular CL content may be dependent on the level of CDP-DG in the cell. In the CL synthase reaction CMP released from CDP-DG can be accounted for by transphosphatidylolation with stoichiometric conversion of PG to CL. It is not known if the intracellular level of CMP may regulate this reaction. However, given the fact that the intracellular CMP pool is approximately 10% of the CTP pool regulation by CMP is rather unlikely. In BHK-21 cells infected with Semliki Forest virus a 70% reduction in cellular CTP was observed [76]. However, biosynthesis of phosphatidylcholine was unaffected. In contrast, when HeLa cells were infected with polio virus a 3-fold increase in cellular CTP level was accompanied by a 2-fold stimulation of phosphatidylcholine biosynthesis [77]. Whether the intracellular CTP level regulated CL biosynthesis in these model systems was not investigated.

Extra-mitochondrial cardiolipin precursors and their import into mitochondria

The movement of phospholipids, and/or their biosynthetic intermediates, from the endoplasmic reticulum to mitochondrial membranes is a central event in mitochondrial membrane biogenesis (for review see [78]). Biosynthesis of PA in rat liver was shown to be bimodal in mitochondria and microsomes [43]. In other studies synthesis of PA was shown to occur on the outer mitochondrial membrane [44, 45]. Thus, it is conceivable that PA could be transported from the outer to inner mitochondrial membrane for synthesis of PG and subsequently CL. In any event PA must be delivered from the endoplasmic reticulum to the mitochondria and/or synthesized in the mitochondria for PG and CL biosynthesis. A rat liver cytosolic protein exchanged [³²P]PA between micro-

somal and mitochondrial membranes [46]. However, mass transport of PA was not demonstrated in this study.

The distribution of CDP-DG synthesis was shown to be bimodal in the endoplasmic reticulum and mitochondria [47, 48, 79]. Total and specific activities in the endoplasmic reticulum membranes appeared to be highest. Recent evidence suggests that rat liver mitochondria was capable of synthesizing CDP-DG on the matrix side of the inner mitochondrial membrane [27, 80]. Studies on the topology of glycerolipid synthesis in microsomal vesicles indicate that the active site of CDP-DG synthase was located exclusively on the cytosolic surface (for review see [83]). Transport of liponucleotides (e.g. CDP-DG) between various membrane systems may occur by spontaneous monomer diffusion due to the polarity of these compounds. However, the exact mechanism of transport of these compounds is unknown. Although preformed CDP-DG's in Guinea Pig microsomal membranes were converted to CL when mixed with mitochondria *in vitro* [49], a preferential utilization from either mitochondria or microsomes *in vivo* is unknown. Since CL is synthesized exclusively in the mitochondria, another important question in modern cell biology is whether extra-mitochondrial pools of PG may be utilized for CL biosynthesis. If this is the case then it is possible that the rate of CL biosynthesis may be regulated by the availability of extra-mitochondrial PG. In spite of the high content of CL and its important role in the maintenance of mitochondrial function and membrane integrity, detailed studies on the control of CL metabolism in the mammalian heart have been largely neglected.

Regulation of cardiolipin biosynthesis in the heart

Regulation of cardiolipin biosynthesis at the level of enzymes of the CDP-DG pathway

PA:CTP cytidyltransferase. PA:CTP cytidyltransferase (E.C. 2.7.7.41) catalyzes the first step of the CDP-DG pathway of CL biosynthesis in the heart [3]. Recent studies in our laboratory have indicated that the PA:CTP cytidyltransferase catalyzes the rate-limiting step of CL biosynthesis in the isolated perfused rat heart [3]. In these experiments hearts were perfused with [1,3-³H]glycerol (pulse labeled) for 5 min followed by perfusion with nonradioactive glycerol (chase) for up to 60 min. Radioactivity was chased out of PA and into PG and CL with time of perfusion (Fig. 3). Based upon these data and the low pool size of CDP-DG in the heart [51], the rate-limiting step of CL biosynthesis in the heart was postulated to be the conversion of PA to CDP-DG. An equivalent amount of PA:CTP cytidyltransferase activity was observed in both the microsomal and mitochondrial fractions prepared from rat heart homogenates. The rate-limiting role of the PA:CTP cytidyltransferase was confirmed by perfusion of

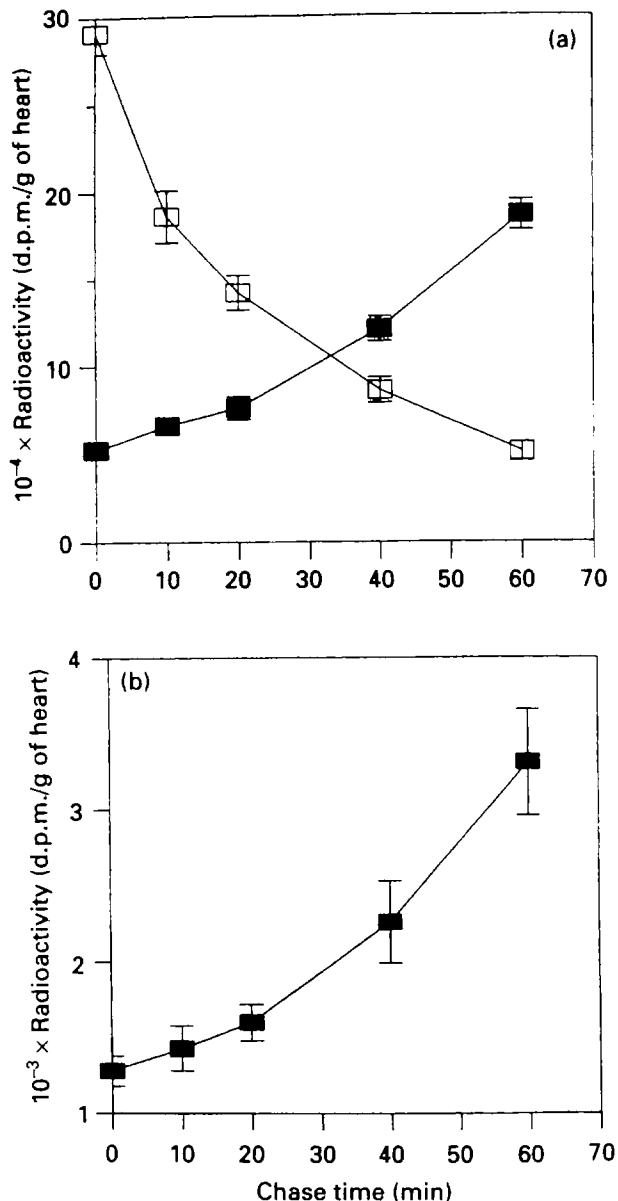


Fig. 3. Pulse-chase study of cardiolipin biosynthesis from [1,3-³H]glycerol. Isolated rat hearts were perfused for 5 min with 0.1 μ M [1,3-³H]glycerol and then perfused for up to 60 min with 0.1 μ M glycerol. Subsequently, the radioactivity in A. PA (open squares) and PG (closed squares) and B. CL was determined. Results represent the mean \pm standard deviation of three hearts.

the heart under conditions which alter the level of CTP in the heart. Hypoxic perfusion of the rat heart reduced cardiac ATP and CTP levels [51]. When hearts were perfused with radioactive glycerol for 60 min under hypoxic conditions the radioactivity incorporated into PG and CL was dramatically reduced compared with controls. A plausible mechanism for the reduction in radioactivity incorporated into PG and CL was that a lowered formation of PA occurred when the ATP levels in the heart were depleted. In addition, a reduced conversion of PA to CDP-DG likely occurred when the cardiac

CTP concentration was limited. The activity of the Triton X-100 solubilized bovine heart mitochondrial PA:CTP cytidylyltransferase has recently been characterized in our laboratory. The enzyme had a pH optimum of 8.5, was activated by phosphatidylcholine, sphingomyelin and lysophosphatidylethanolamine, inhibited by lysophosphatidylcholine and was found to be heat and acid labile (Taylor WA, Hatch GM, unpublished observations).

Phosphatidylglycerolphosphate synthase. PGP synthase (E.C. 2.7.8.5) catalyzes the second step of the CDP-DG pathway of CL biosynthesis in the heart, a reaction involving the condensation of CDP-DG with *sn*-glycerol-3-phosphate to form PGP [3]. In a Chinese hamster ovary mutant of phosphatidylinositol biosynthesis, PG was substituted for phosphatidylinositol [81]. This PG was synthesized in the endoplasmic reticulum. In addition, PG is synthesized extensively in the endoplasmic reticulum in the rabbit lung [82]. In the rat heart the majority of PGP synthase activity was localized to the mitochondria with less than 10% of this activity observed in microsomes [56]. The mitochondrial enzyme had a neutral pH optimum and required magnesium for activation. The enzyme was heat labile as preincubation at 55°C for 5 min abolished 70% of the original activity. The apparent K_m 's for *sn*-glycerol-3-phosphate and CDP-DG were calculated to be 20 and 46 μ M, respectively. As mentioned previously thyroid hormone treatment of rats was shown increase the CL content of the heart [35]. If cardiac CL mass was increased in mitochondria of thyroid hormone treated animals then it would be logical to surmise that the biosynthesis of PG should also be increased to provide substrate for the increased demand of CL biosynthesis. We investigated if thyroxine affected the biosynthesis of PG in isolated rat heart mitochondrial fractions. Rats were injected with thyroxine (250 μ g/Kg) for 5 days and the mass of cardiac PG in the mitochondria determined in these animals. Treatment with thyroxine did not affect the body weight but resulted in a 30% increase in freeze-dried ventricular weight compared with sham injected controls [52]. The ratio of ventricular weight to body weight was increased from 0.64 to 0.89 ($\times 1000$) in the thyroxine-treated animals. Thyroxine treatment resulted in a 34% increase in mitochondrial PG content in these animals. The mechanism for this increase in PG synthesis was determined to be a striking 3.5-fold increase in PGP synthase activity. The activities of PA:CTP cytidylyltransferase and PGP phosphatase were not affected. Thus, PGP synthase activity in the heart may be regulated by hormonal intervention.

Phosphatidylglycerolphosphate phosphatase. PGP phosphatase (E.C. 3.1.3.27) catalyzes the conversion of PGP to PG in the heart [3]. In the heart the majority of PGP phosphatase activity was localized to the mitochondria with less than 10% of this activity observed in microsomes [56]. The enzyme had

a pH optimum of 6.5, was inhibited by high concentrations of PGP and was extremely heat labile as pre-incubation at 55°C for 5 min abolished 95% of the original activity. Previous studies had set a precedent for the modulation of PGP phosphatase activity by lipids [53]. PA was shown to both inhibit and stimulate PGP phosphatase activity in rat liver mitochondria depending upon the form (sodium or ammonium salt) of the phospholipid presented to the enzyme [53]. The heart has a tremendous capacity to take up and utilize fatty acids for phospholipid and triacylglycerol biosynthesis and β -oxidation (for comprehensive reviews see [54, 84]). Extraction ratios of up to 70% have been measured during one single transit of blood or perfusion fluid through the capillary system. The presence of stearic acid in the perfusate of isolated hamster hearts stimulated the incorporation of radioactive choline into phosphatidylcholine [55]. This prompted us to examine whether fatty acids influenced cardiac CL biosynthesis. Rat heart mitochondrial fractions were isolated and PGP phosphatase activity was determined in the absence or presence of various fatty acids. The presence of 0.5 mM oleic acid was found to markedly stimulate cardiac mitochondrial PGP phosphatase activity 1.9-fold compared with controls [56]. In contrast, higher concentrations (1.0–2.0 mM) attenuated the activity of the PGP phosphatase. This may have been due to alterations in the enzyme lipid associations required for enzyme catalysis. Further studies indicated that only unsaturated fatty acids were effective in stimulating PGP phosphatase activity *in vitro* and that an anionic bile detergent, taurocholate, was without effect. Remarkably, when mitochondrial fractions were pre-incubated at 55°C followed by assay for the remaining activity, oleic acid appeared at first to retard the heat inactivation of the PGP phosphatase. However, because the rate of inactivation of PGP phosphatase in both control and oleic acid containing incubations was identical, attenuation of enzyme denaturation was unlikely. Fatty acids are associated with fatty acid binding proteins *in vivo* [57]. Since oleic acid bound to albumin also stimulated the PGP phosphatase a potential regulation of the activity of PGP phosphatase *in vivo* was postulated [56]. We investigated this directly in hearts perfused with [1,3-³H]glycerol in the absence or presence of physiological and pathophysiological concentrations of fatty acid. When hearts were perfused with a 1:1 mM ratio of oleic acid bound to albumin CL biosynthesis was stimulated 3.1-fold compared to controls [58]. However, when hearts were perfused with a 2:1 mM ratio of oleic acid bound to albumin the stimulatory effect of oleic acid on CL biosynthesis was attenuated. Since the pool sizes of CL and its precursors were unaltered in these studies it was possible that an *in vivo* attenuation of new CL biosynthesis under pathophysiological plasma concentrations of fatty acid had occurred. Such elevated plasma concentrations of fatty acids have been observed in the nephrotic syndrome and uncontrolled diabetes.

In addition, elevated plasma fatty acid levels are observed following acute myocardial infarction or during cardiac surgery and ischemic cardiac muscle is exposed to these during reperfusion (for review see [84]). This raises the possibility that CL biosynthesis may be attenuated during ischemia-reperfusion. However, direct evidence for this is lacking.

Cardiolipin synthase. CL synthase catalyzes the formation of CL from PG and CDP-DG in the heart [3]. CL synthase activity was localized exclusively to the mitochondrial fraction. The heart enzyme had a pH optimum of 8.5 and required cobalt for maximum activity. The small amount of enzyme activity (3.1 ± 0.8 pmole/min/mg mitochondrial protein) observed in the heart might suggest that this enzyme is rate-limiting for CL biosynthesis. Indeed, as previously discussed, Hostetler [34] demonstrated that thyroid hormone treatment of rats stimulated CL synthase activity 52% in rat liver mitochondria and suggested that the increase in CL synthase activity may have been responsible for the observed increase in CL mass in the livers of thyroid hormone treated rats [32]. We examined the effect of thyroid hormone on CL synthase activity in isolated rat heart mitochondria. When rats were treated with thyroxine (250 μ g/Kg body weight) for 5 days and cardiac mitochondrial CL synthase activity was examined, a 2.8-fold increase in CL synthase activity compared to controls was observed [52]. In addition, the increase in cardiac CL synthase activity was associated with a 23% increase in mitochondrial CL mass. The difference in CL synthase activity between that of the liver [34] and the heart [52] may be related to the fact that the heart contains greater than 2.6-fold the CL content of the liver [2], when expressed on the basis of percent total phospholipid phosphorus, and may thus require a higher induction of enzymatic activity in order to produce the required amount of cardiac CL in the thyroid hormone-treated animals.

Regulation of cardiolipin biosynthesis at the level of substrate precursors

Exogenous phosphatidylglycerol may be utilized for cardiac cardiolipin biosynthesis. PG is the immediate precursor of CL biosynthesis in mammalian tissues [23, 26]. PG was shown to be released from liver perfusates and this pool of PG was postulated to be involved in the attenuation of platelet aggregation [19]. This prompted us to examine if exogenous PG could be utilized for CL biosynthesis in the heart. Hearts were perfused with radioactive PG bound to albumin for up to 30 min and the radioactivity incorporated into CL determined. PG was rapidly, within 5 min, taken up by the isolated heart and the uptake of intact PG was confirmed using a fluorescent analog of PG [59]. The uptake of radioactive PG was unaffected by the presence of 1–50 μ M PG in the perfusate. Concentrations of PG above 50 μ M attenuated radioactive

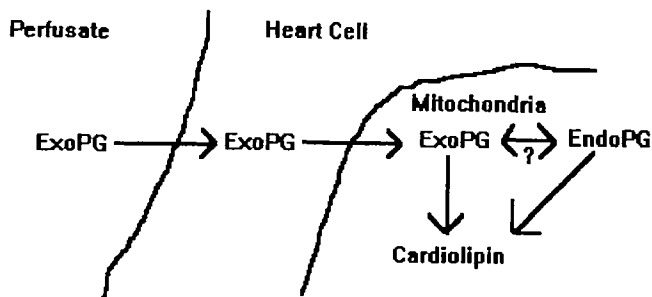


Fig. 4. Model of cardiolipin biosynthesis from exogenous phosphatidylglycerol in rat heart. ExoPG, exogenous PG; EndoPG, endogenous PG. See text for description.

PG uptake (unpublished data). A significant amount of radioactive PG was converted to CL in a time-dependent manner. In addition, formation of some radioactive lysoPG was also observed. Formation of this lysoPG was attributed to the presence of phospholipase A activity in the mitochondrial, microsomal and cytosolic fractions. A model was proposed in which PG is rapidly taken up by the heart, then readily enters into the mitochondria where it becomes available for CL biosynthesis (Fig. 4). Whether the exogenous extra-mitochondrial pool of PG mixes with endogenous pre-existing or endogenous newly synthesized PG in the cardiac mitochondria is unknown. Nevertheless, these studies indicated that in addition to endogenous PG synthesized within the heart, exogenous (extra-mitochondrial) pools of PG may be utilized for CL biosynthesis [59]. Since CL biosynthesis occurs exclusively in the mitochondria, a potential regulatory role of PG synthesized outside the mitochondria (i.e. endoplasmic reticulum) on CL biosynthesis was indicated.

Distinct pools of phosphatidylglycerol within the heart are available for cardiac cardiolipin biosynthesis. Diabetes in rats is associated with alterations in cardiac CL. In cardiac sarcolemmal membranes the mass of CL was shown to be decreased [60], whereas in the cardiac sarcoplasmic reticulum of these animals the CL mass was increased [61]. We examined cardiac polyglycerol phospholipid mass in STZ-induced diabetic rats. The ventricular pool sizes of CL and most other phospholipids were unaltered in the hearts of STZ-induced diabetic rats [62]. These studies indicated that the observed alterations in cardiac CL mass in sarcoplasmic reticulum [61] and sarcolemmal membranes [60] of STZ-induced diabetic rats were likely due to altered distribution of CL within the heart and not an alteration in overall cardiac CL mass. In addition, the activity of the enzymes of the CDP-DG pathway were not affected by diabetes. However, a striking 36–40% decrease in the pool size of ventricular PG was observed in diabetic hearts compared with controls [62]. A time course study revealed that this had occurred rapidly within 24 h post injection of streptozotocin. The reduction of PG mass was observed in both the $10,000 \times \text{g}$ and $100,000 \times \text{g}$ membrane

fractions. When isolated hearts from these groups were perfused for up to 30 min with [^{32}P]inorganic phosphorus there was no difference in the amount of radioactivity incorporated into PG or CL. Since radioactivity associated with PG was unaltered, a calculated 1.8-fold increase in the specific radioactivity of PG was observed in the hearts of diabetic rats compared with controls. Since the radioactivity incorporated into CL and the rate of CL biosynthesis were unaltered, as indicated by a lack of alteration in the activity of the enzymes of the CDP-DG pathway, it was postulated that CL was synthesized from newly synthesized PG [62]. These studies indicated the existence of distinct pools of PG within the heart and indicated that the newly synthesized pool of PG used for CL biosynthesis does not appear to immediately mix with the pre-existing pool of PG in the heart.

Future perspectives

A number of significant advances have been made over the past several years on the elucidation of the mechanisms which govern CL biosynthesis in the heart. However, several fascinating questions remain. With respect to the biosynthetic enzymes, the mechanism for the modulation of their activities remains undefined. The regulatory properties that govern the PA:CTP cytidyltransferase, PGP synthase, PGP phosphatase and CL synthase are only now beginning to emerge. Clearly, purification of these enzymes from the mammalian heart will be a major contribution to the field. Are these enzymes regulated by transcription, translation or post-translational modification? The role and regulation of the resynthesis of lysoPG as a CL precursor, monolysoCL and possibly dilysoCL for the remodeling of cardiac CL are slowly being uncovered. These are important questions since cardiac CL is highly enriched in 18:2 and 18:3 molecular species and these may be influenced by dietary manipulation. The role of co-factors and precursor substrates (e.g. CTP, CDP-DG availability) in the regulation of CL biosynthesis in the heart remains undefined. Clearly, the elucidation of these and other control mechanisms is essential for the development of our understanding of how CL biosynthesis is regulated in the heart. Since the turnover of CL is relatively slow compared with that of the other major membrane phospholipids there must be levels of coordination between biosynthesis and catabolism of this important phospholipid. The elucidation of these metabolic processes represent a major future challenge for scientists interested in studying CL metabolism in the heart.

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