Chapter 23
Alterations in Phospholipase D During the Development of Myocardial Disease

Paramjit S. Tappia and Naranjan S. Dhalla

Abstract Phospholipase D (PLD) produces phosphatidic acid, which is converted to diacylglycerol (DAG) by phosphatidate phosphohydrolase (PAP). Since both these lipid signaling molecules regulate Ca^{2+}-movements, they also influence cardiac contractile function. In this article, we discuss the importance of PLD in relation to the production of lipid signaling molecules and regulation of cardiac function under various pathophysiological conditions such as ischemic heart disease, diabetic cardiomyopathy, and congestive heart failure. In fact, marked alterations in PLD activities have been reported to occur in ischemic heart, diabetic heart, and failing heart. While the mechanisms of changes in PLD activities in heart disease may be of complex nature, oxidative stress seems to play a critical role in the activation of PLD. From the evidence provided it is suggested that impairment in this phospholipid signal transduction pathway results in cardiac dysfunction during the development of different myocardial diseases.

Keywords Phospholipase D • Signal transduction • Diabetic cardiomyopathy • Congestive heart failure • Ischemia-reperfusion injury • PLD-mediated signal transduction

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23.1 Introduction

The hydrolysis of phosphatidylcholine (PC) by phospholipase D (PLD) produces phosphatidic acid (PA), which in turn is converted to 1, 2 DAG by the action of phosphatidate phosphohydrolase (PAP) [1, 2]. Both PLD and PAP are thus considered to modulate myocardial levels of PA and DAG. Different hormones such as norepinephrine, endothelin-1, and angiotensin II (Ang II) have been shown to increase formation of PA in cardiomyocytes [3, 4] and stimulate sarcolemmal (SL) and sarcoplasmic reticular (SR) Ca^{2+}-transport systems [5, 6]. Furthermore, PA has been reported to increase the intracellular concentration of free Ca^{2+} in adult cardiomyocytes and to augment cardiac contractile activity of the normal heart [5, 7]. DAG can also influence cardiac function through phosphorylation of myocardial proteins, including ion channels, via activation of protein kinase C (PKC) isozymes [8]. These PLD-mediatd signal transduction events are summarized in Fig. 23.1.

Two mammalian PLD isozymes, PLD1 and PLD2, have been cloned [9]. While PLD1 is localized to the Golgi apparatus and nuclei [10], PLD2 is the major myocardial PLD isozyme specifically localized to the SL membrane [11]; other subcellular localizations of PLD2 have also been reported [12, 13]. Interestingly, a transient expression of PLD1 during heart development in rats has been demonstrated [14]. In this regard, the level of PLD1 protein increased transiently from 0 to 3 days postpartum and declined gradually beginning 7 days after birth. This suggested that PLD1 protein in the heart is strongly associated with the early postnatal development of the heart in rats [14].

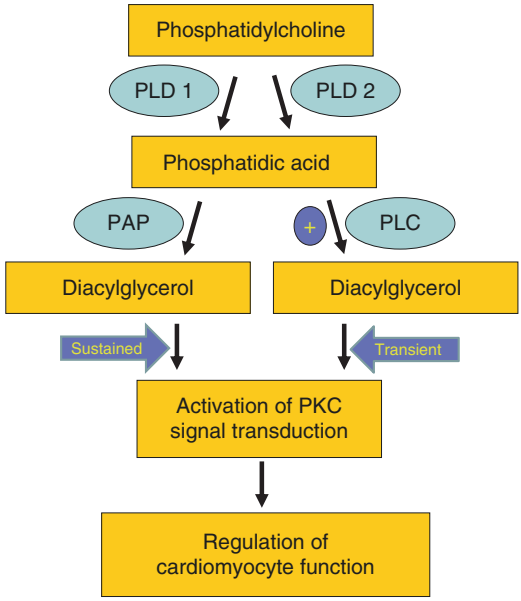

Fig. 23.1 Myocardial phospholipase D signal transduction. *PLD1* phospholipase D1, *PLD2* phospholipase D2, *PAP* phosphatidate phosphohydrolase, *PLC* phospholipase C, *PKC* protein kinase C; +, stimulation

PLD1 requires phosphatidylinositol 4, 5-bisphosphate (PIP_2) for its activity, which is stimulated by PKC and Rho small G-protein family members [9, 15–24]. PLD2 also requires PIP_2 for its activity [11], but, unlike PLD1, PLD2 is activated by unsaturated fatty acids [2, 16, 17, 25, 26] and is insensitive to the PLD1 activating factors [27]. It should be noted that PLD isozymes contain N-terminal PH (pleckstrin) and PX (phox) homology domains. Both these domains also interact specifically with distinct phosphoinositide ligands [28]. Both the PH and PX domains are important for PLD function by controlling the dynamic association of the enzyme with the plasma membrane. Thus, there are two modes of PLD regulation by phosphoinositides; stimulation of activity mediated by the polybasic domain and dynamic regulation of membrane targeting mediated by the PH and PX domains [28].

Some studies have shown that both receptor- and non-receptor coupled tyrosine kinases are involved in the regulation of PLD activity, in addition to serine/threonine kinases, Ca^{2+}-calmodulin-dependent protein kinase, and cAMP kinases [29–31]. G-proteins, Gα12 and Gα13, have also been reported to activate PLD [32]. Another important regulator of PLD is ARF; ARF directly activates PLD1 and has also been shown to activate PLD2 [25, 33–36]. In fact, PLD2 has been reported to be selectively activated by ARF6 [12]. It is interesting to note that U73122, a known phospholipase C inhibitor, is a potent inhibitor of myocardial PLD by a PIP_2-dependent mechanism and thus PLD may be involved in some of the effects ascribed to PLC [37]. While there is some information on the posttranslational mechanisms of regulation of the myocardial PLD isozymes, this is not completely understood.

The increased formation of reactive oxygen species (ROS) is generally associated with oxidative stress and subsequent cardiovascular injury and cardiac dysfunction [38–40]. Since ROS and oxidant molecules such as H_2O_2 are implicated in the pathogenesis of cardiac dysfunction, this article is intended to describe the role of oxidative stress in relation to myocardial PLD and cardiac dysfunction under different myocardial diseases such as diabetic cardiomyopathy, congestive heart failure, and ischemic heart disease.

23.2 Impairment of PLD Activities During Diabetes

Oxidative stress has been implicated in the pathogenesis of diabetic cardiomyopathy [41–47]. As a consequence of the effects of oxidative stress on the cardiomyocytes, it would be expected that oxidants and ROS could have an impact on the PLD activity during diabetes. In fact, SL PLD activities have been shown to be significantly depressed in diabetic animals [48, 49], resulting in a marked reduction of PLD-derived PA. It has been suggested that this could lead to an impairment of cardiac function in chronic diabetes [48, 49].

It is pointed out that enhanced tissue Ang II levels have been reported in diabetes and might lead to cardiac dysfunction through oxidative stress [50]. Recently Ang II-induced NADPH oxidase has been shown to be involved in hyperglycemia-induced cardiomyocyte dysfunction, which might play a role in diabetic cardiomyopathy [51] and may be related to impaired PLD activities due to superoxide

generation. Impaired PLD activation has been shown to be involved in the damaging effects of oxidative stress in other cells as well. Decreased superoxide generation by neutrophils in insulin-dependent diabetics is, in part, due to impaired activation of PLD [52], and is solely due to high glucose concentrations. The suppressive effect of glucose on diabetic neutrophils is associated with a reduction in PLD activation, which improves when diabetic neutrophils are placed in a normal glucose environment. Glucose causes a reduction in PLD activation, leading to a decrease in second messenger generation and incomplete activation of the respiratory burst [52]. It is interesting to note that we have reported a decrease in the SL amount of PIP_2, due to depressed activities of the phosphatidylinositol (PI) kinases in the diabetic heart [53], likely as a result of oxidant-mediated depression in the PI kinase activities [54]. In this regard, the depressed SL PLD activity during diabetes [45, 46] may also be explained on the basis of a reduced SL PIP_2 level. While direct information on the redox regulation of PLD isozyme activities and the functional consequences of changes in PLD activities in diabetic cardiomyopathy remains to be established, it is reasonable to assume that the depressed PLD activities in the heart during diabetes may be due to oxidative stress.

23.3 Abnormal PLD Activities During Cardiac Hypertrophy and Heart Failure

It is well known that heart failure is a major cause for significant morbidity and mortality; however, the pathophysiological events have not been fully elucidated. There is growing evidence that oxidative stress is implicated in the cardiac dysfunction leading to CHF [55–58]. Oxygen-free radicals can affect heart SL [59–62], SR [63], and mitochondrial functions [64], thus affecting signal transduction mechanisms that are possibly involved in cardiac remodeling and subsequent CHF. Since oxidative stress has significant effects on the SL membrane during CHF, it can be assumed that the oxidative stress will also exert detrimental effects on PLD activities.

The mRNA expression levels of both PLD1 and PLD2 have been reported to be markedly enhanced in ventricular pressure-overload hypertrophy subsequent to aortic banding in rats [65]. A similar induction of PLD mRNA and protein expression has also been reported in hypertrophied human hearts of individuals who had died from noncardiac causes [65]. These authors suggested that PLD activation by α-adrenoceptor and PKC plays a significant role in cell signaling in hypertrophy due to pressure overload [65]. Ventricular fibrosis is promoted by many factors that activate PLD and induce cardiac dysfunction and heart failure. In a hypertensive heart failure model using Dahl-Iwai salt-sensitive rats, PLD activity was seen to be increased with progressive ventricular fibrosis, leading to myocardial stiffening and heart failure [66]. Inhibition of PLD activity with administration of *N*-methylethanolamine decreased collagen content, prevention

of myocardial stiffening, attenuation of ventricular hypertrophy as well as hemodynamic deterioration [66].

We have previously shown that PLD activities are differently altered in CHF subsequent to myocardial infarction induced by the occlusion of the coronary artery [67]. While SL PLD1 activity was decreased, an increase in PLD2 activity was observed in the viable left ventricular tissue. Although the specific role of cardiac PLD isozymes is not fully established, an oleate-dependent PLD activity has been shown to be drastically increased during apoptosis of Jurkat T cells [68], whereas increased PLD2 activity has been shown to reduce hypoxia-induced death of PC12 cells [69]; these studies suggest that PLD2 may play a role in cellular apoptosis. It is interesting to note that Ang II activates NADPH oxidase [70, 71], which can be prevented by imidapril, a known angiotensin converting enzyme inhibitor. Activation of the renin-angiotensin system is the hallmark of CHF [72]. In addition, increased myocardial NADPH oxidase activity in CHF has been reported [73, 74]. We have earlier shown that imidapril normalizes the augmented PLD2 activity in CHF [75]. It is possible that this may be due to a blockade of NADPH oxidase and ROS-mediated activation of PLD2. However, while extensive studies need to be conducted to fully determine the functional significance as well as the mechanisms of impaired PLD1 and PLD2 activities in CHF, it is likely that PLD isozymes are altered due to oxidative stress and may influence cardiomyocyte function of the failing heart through impaired Ca^{2+}-handling.

23.4 Alterations in PLD Activities During Cardiac Ischemia-Reperfusion

A decrease in the blood supply to the heart due to atherosclerosis, thrombosis, or coronary artery spasm is known to induce myocardial ischemia. Although reperfusion of the ischemic myocardium during early stages is essential to prevent cardiac damage, reperfusion of the ischemic heart, after a certain critical period, exerts deleterious effects. These are represented by contractile dysfunction, an increase in infarct size, ultrastructural damage, and changes in myocardial metabolism, which at a later stage leads to cell necrosis [76]. During ischemia, mitochondrial carriers are in a reduced state, due to the degradation of the adenine nucleotide pool. Thus, the interaction of molecular oxygen trapped within the inner membrane of the mitochondria with the leakage of electrons from the respiratory chain leads to the formation of ROS [77]. The deleterious effects of oxidative stress in myocardial I-R are well documented and strongly correlated with cardiac dysfunction [78], a decrease in the antioxidant defense mechanism [79, 80] as well as an increase in lipid peroxidation [80, 81], leading to increased membrane permeability. PLD has been shown in many cases to contribute to the deleterious effects due to oxidative stress in I-R injury. For example, lipid oxidation products such as oxidized LDL have been considered prime candidates for inducing cellular necrosis. Oxidized LDL stimulates PLD [82], implicating a role for PLD in cellular necrosis. Cardiac SL sodium-hydrogen (Na^+–H^+)

exchanger is critical for the regulation of intracellular pH and its activity contributes to I-R injury. Incubation of porcine cardiac SL vesicles with exogenous PLD results in an inhibition of Na^+–H^+ exchanger [83]. It was concluded that PLD-induced changes in the cardiac SL membrane phospholipid environment alter Na^+–H^+ exchanger activity.

While some investigators have reported that the activation of PLD is associated with an improvement of post-ischemic functional recovery and attenuation of cellular injury [84], other investigators, as well as work from our laboratory, have found that PLD is not activated in the ischemic heart [85–88]. Furthermore, our studies also revealed that the increase in the SL PLD2 activity in early reperfusion of the 30 min ischemic heart was associated with an increase in V_{max}, indicating that the PLD2 activation may be due to posttranslational modifications as a result of oxidative stress. On the other hand, we have reported that a Ca^{2+}-independent phospholipase A_2 (cytosolic PLA_2) and subsequent mobilization of the unsaturated fatty acid has been shown to modulate the activity of PLD in heart SL [89]. Interestingly, the cytosolic PLA_2 is also activated by H_2O_2 [90], which could provide a mechanism of an indirect regulation of the SL PLD2 activity by H_2O_2. It should be noted that we also observed a decrease in the SR PLD2 activity after 5 min of reperfusion. Although the K_m value of the SR PLD2 was reduced (increased substrate affinity), the depressed V_{max} value would seem to imply a defect in the catalytic domain of this enzyme; it was suggested that a reversible oxidation may occur since the PLD2 activity was recovered after 30 min reperfusion. In fact, SR PLD activity, in vitro, has been reported to be inhibited by nonradical oxidants, H_2O_2 and HOCI, through reversible modification of associated thiol groups [18]. Thus, the enzyme may be controlled by the GSH redox status of the cardiac cell. In this regard, in the isolated perfused rabbit heart, an ischemic period results in a progressive reduction of tissue glutathione content and of the GSH/GSSG ratio [91], while post-ischemic reperfusion has been shown to lead to a further decrease in the GSH/GSSG ratio [91]. However, a similar response has also been demonstrated for the SL enzyme [92], which is not consistent with the increase in its activity. This inconsistency could be explained on the basis that the functional thiol groups of the SL PLD2 in the isolated perfused heart are not as readily accessible by oxidants as these are in the isolated SL preparation. Such differences may exist between the sensitivity of the SR and SL PLD to different concentrations of oxidant molecules as well as ROS.

Ischemic preconditioning (IP) involving a brief period of ischemia, prior to a prolonged period of ischemia, has been shown to improve myocardial function and diminishes the infarct size. Activation of PLD due to I-R injury as well as in the preconditioned hearts has been documented [93–95]. Agonists of PLD simulate the effects of IP, whereas the inhibition of PLD blocks the beneficial effects of IP as evidenced by the increased incidence of ventricular arrhythmias [85]. The inhibition of PLD can be seen to reduce the amount of DAG and PA as well as significantly inhibit the stimulation of PKC. Thus, PLD may play a role in the myocardial protection afforded by IP. Indeed, this protective effect may be due to ROS generation during the IP [96, 97], which may also be related to the activation of PLD, thus providing a mechanism of action of IP and protection against I-R injury. In addition,

myocardial adaptation to ischemia (IP) is considered to occur through the activation of several tyrosine kinases [98]. The phosphorylation of tyrosine kinases has been shown to be linked with the activation of PLD leading to the activation of multiple kinases [93, 94] including PKC isozymes [99], therefore suggesting that PLD may be a component in the redox signaling designed to protect the heart during IP. While the exact consequences of the changes in PLD1 and PLD2 activities in the heart remain to be determined, PLD isozymes could emerge as an important target for protection against injury during cardiac I-R.

23.5 Conclusions

From the aforementioned discussion, it is evident that impairment of myocardial PLD activities is associated with cardiac dysfunction under different myocardial diseases, while PLD isozyme specific activation may provide cardioprotection (Fig. 23.2). Although significant advancements have been made, more is required to define the role of PLD in different cardiac pathologies. While oxidative stress appears to be a major factor in causing PLD abnormalities, the targeting of PLD, more specifically, modulation of membrane PA levels, may offer a potential for drug development. Defects in other phospholipid-mediated signaling pathways (PLC and PLA2) are also implicated in different myocardial diseases, and in view of the cross-talk and complexities between these pathways (Fig. 23.3), lipid products

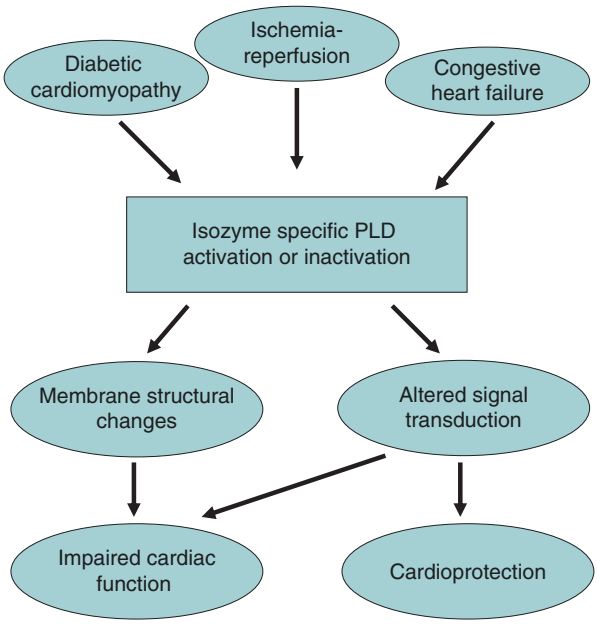

Fig. 23.2 Differential changes and impact of phospholipase D during different cardiac pathologies. *PLD* phospholipase D

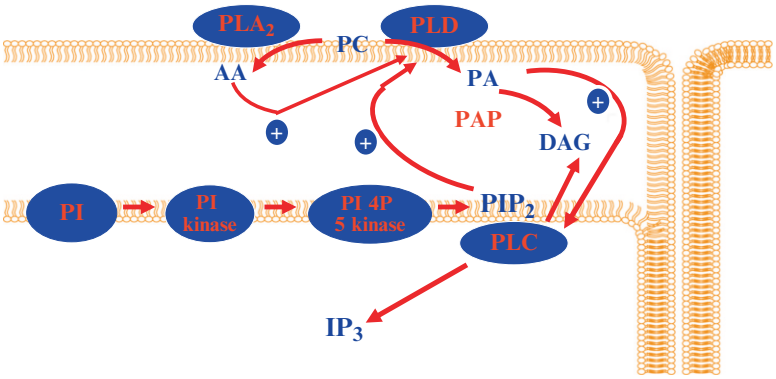

Fig. 23.3 Complexities of phospholipid-mediated signal transduction pathways. *PLA_2* phospholipase A_2, *PLD* phospholipase D, *PAP* phosphatidate phosphohydrolase, *PLC* phospholipase C, *PC* phosphatidylcholine, *DAG* diacylglycerol, *PA* phosphatidic acid, *AA* arachidonic acid, *PI* phosphatidylinositol, *PI4P* phosphatidylinositol-4 phosphate, *PIP_2* phosphatidylinositol,-4,5-bisphosphate, *IP_3* inositol-1,4,5-trisphosphate; +, stimulation

generated through their activities may not only alter signal transduction processes, but also modulate the lipid microenvironment of membrane-associated proteins. Thus, alterations in the PLD activities can be seen to influence cardiac function and may constitute additional therapeutic targets for drug discovery [100–102] for the treatment of heart disease due to different etiologies.

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