Numerous studies have shown that lipid peroxidation is involved in the pathogenesis of various degenerative diseases including atherosclerosis, diabetes, cancer, inflammation, drug-induced toxicity, and neurodegenerative disease. Protein kinase C (PKC), a lipid activated kinase, is a key enzyme for intracellular signaling pathway in physiological conditions. Recently considerable evidence has accumulated to confirm that lipid peroxidation participates in intracellular signal transduction in the pathophysiology of aerobic organisms. Lipid peroxides, such as hydroperoxy fatty acids, oxidized cholesteryl linoleates and 4-hydroxy-2-nonenal, efficiently stimulate PKC in the cells. More importantly, oxidized diacylglycerol strongly stimulates PKC as much as phorbol ester, a strong tumor-promoting PKC activator. This review article describes PKC signaling pathway, the role of lipid peroxides in the intracellular signaling, the significance of lipid peroxidation in pathophysiology, and the cellular defense systems against oxidative stress.

**Key words:** lipid peroxidation, oxidative stress, protein kinase C, diacylglycerol

### I. Introduction

Lipid peroxidation, an oxygen radical-initiated event, evokes a degradative process in cells and tissues, which has been implicated in the pathogenesis of various diseases including atherosclerosis, ischemia/reperfusion injury, diabetes, inflammation, drug-induced toxicity, cancer and neurodegenerative disease [7, 11, 16, 17, 28, 38, 41, 42]. Moreover, it has been well documented that lipid peroxides can alter the signal transduction through the modification of cell-signaling molecules, and have functional consequences [14, 22, 43].

Protein kinase C (PKC) plays a crucial role in receptor-mediated signal transduction affecting a diverse range of cellular responses such as cell proliferation, differentiation, hormonal action and neurotransmission [2, 3, 6, 29–31]. On the other hand, various reports indicate that PKC molecules also participate in pathological processes such as tumor promotion, inflammation, autoimmune diseases, atherosclerosis and neurodegenerative disorder [10, 15, 23, 36, 37]. Recently, many reports present considerable evidence that oxidative stress increases activity of PKC signaling pathways [19, 32, 35, 40, 45]. This review described the roles of oxidative stress including lipid peroxidation in intracellular PKC signaling with regard to the significance of oxidative stress in pathophysiological events. In addition, cellular defense systems corresponding to reactive oxygen species were discussed.

### II. PKC

PKC takes a part in cellular responses to various agonists including hormones, neurotransmitters and growth factors (Scheme 1). PKC is a unique enzyme which is activated by calcium ion and lipids such as phosphatidylserine (PS), 1,2-diacylglycerol (DAG), free fatty acids (FFA), and lysophosphatidylethanolamine. PKC represents a family of more than 11 phospholipid-dependent serine/threonin kinases. The isoforms are divided into the following three groups according to their sensitivity toward the activators. Conventional PKCs
are calcium-dependent, and stimulated by DAG. Novel PKCs are calcium-independent, but are also diacylglycerol (DAG) stimulatable. Atypical PKCs require neither calcium nor DAG for optimal activity. A variety of reports demonstrate that individual PKCs mediate different biological processes in the cell. The exact role of individual PKCs is now being elucidated. PKCs are the major cellular target for activation by tumor-promoting phorbol esters, and consequently, PKCs are thought to play an important role in carcinogenesis [1, 4, 24, 31]. On the other hand, Mattson et al. reported that the over-activation of PKC induced by phorbol ester may cause the neuronal cell degeneration through the over-phosphorylation of microtubule-associated protein, MAP2 (tau) [25]. Phorbol esters mimic the action of DAG in regard to binding to specific motifs within the PKC regulatory domain, and strongly activate PKC molecules. Production of DAG in response to physiological stimulators is transient due to the rapid conversion of DAG in the cell. In contrast to DAG, phorbol esters are metabolically stable in most cells. Consequently, PKC activation by phorbol esters is much more prolonged than the transient activation that occurs with DAG. Prolonged PKC activation of the phorbol esters is significant in tumor promotion and cell injury [13]. Although phorbol ester, a powerful PKC activator, evokes a variety of disorders via over-activation of PKC, it is an artificial substance and never exists naturally in mammalian cells.

III. Reactive Oxygen Species (ROS) and PKC Signaling

ROS are reactive by-products in aerobic organisms which utilize oxygen molecules for cellular metabolism. Superoxide anion radical (O$_2^-$), hydroxyl radical (·OH) and singlet oxygen (¹O$_2$) are the main cellular free radical species. Though hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO-) and lipid hydroperoxide (LOOH) are not free radicals, they contribute importantly to cellular redox potential, signal transduction and pathological processes (Scheme 2). There is considerable evidence that ROS increases activity of PKC signaling in a variety of cellular functions. Konishi et al. reported that the treatment with H$_2$O$_2$ enhances enzyme activity of PKC δ in COS-7 cells [21]. In this experiment,
phosphorylation of tyrosine residues, which is conserved in the catalytic domain of PKC δ, is critical for PKC activation induced by \( \text{H}_2\text{O}_2 \) action. In addition, \( \text{H}_2\text{O}_2 \) directly induces oxidative modification of PKC, and thereby effectively activates the enzyme [12].

IV. Activation of PKC by Lipid Peroxides

It is well known that the unsaturated fatty acid components of membrane lipids, which are susceptible to oxidation in vivo and in vitro, are readily oxidized to produce hydroperoxy fatty acids, a kind of lipid peroxide. Hydroperoxy fatty acid derivatives of oleic, linoleic, or arachidonic acid stimulate the activity of PKC more efficiently than unoxidized FFA [35]. This result suggests that mere lipid peroxides become the second messenger in the case of PKC signaling which is activated by a lipid mediator. In addition, a variety of by-products in lipid oxidation reaction such as oxidized cholesteryl linoleates, 4-hydroxy-2-nonenal, cardiolipin hydroperoxide and phosphatidylserine hydroperoxide have received considerable attention [14, 18, 22, 34, 43]. Oxidized cholesteryl linolate is shown to specifically induce the endothelial cells to bind monocytes via activation of PKC, extracellular signal-regulated kinase (ERK) and ERK kinase (MEK). 4-Hydroxy-2-nonenal, which is a major end product in oxidized fatty acid metabolism, strongly induces phosphorylation of c-Jun N-terminal kinase (JNK) of PKC, extracellular signal-regulated kinase (ERK) and ERK kinase (MEK). 4-Hydroxy-2-nonenal, which is a major end product in oxidized fatty acid metabolism, strongly induces phosphorylation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase in rat liver epithelial cells [32]. Furthermore, PKC β mediates 4-hydroxy-2-nonenal-induced release of chemotactic protein-1 in murine macrophages [32]. These results clearly suggest a functional relationship between PKC and these oxidative by-products. Exposure of cells to 4-hydroxy-2-nonenal can also lead to the growth inhibition, alteration in enzymatic activities and inhibition of protein synthesis. These effects are the result of the high nucleophilic properties of 4-hydroxy-2-nonenal, which react with electrophilic sites, such as amino and thiol group of proteins.

More importantly, we demonstrated that 1,2-diaclyglycerol hydroperoxide (DAG-OOH) activated rat brain PKC as efficiently as phorbol ester [40]. In addition, DAG-OOH induced \( \text{O}_2^- \) formation from human neutrophils as much as phorbol ester [45]. These results markedly suggested that DAG-OOH might act as a biological messenger in oxidative stress to efficiently alter the PKC-dependent signal transduction system to a similar extent as PMA (Scheme 3).

Recently we also showed that phospholipase C (PLC) hydrolyzed phosphatidylcholine hydroperoxides in the liposomal membrane to DAG-OOH [19]. Moreover, Yamamoto et al. recently succeeded in detecting DAG-OOH in the liver that resulted in the development of acute hepatitis, and later hepatic cancer in LEC rats having accumulated copper in the liver (unpublished data).

V. Roles of Anti-Oxidative Enzymes as Preventive Agents

Oxidative stress refers to the consequences of imbalance between the production of ROS and the ability of the cells to defend against ROS. Cellular defense systems exist to counterbalance ROS in aerobic organisms. The systems consist of enzymatic and non-enzymatic antioxidants that lower the steady-state concentration of ROS and repair the oxidative cell injury. \( \text{O}_2^- \) is commonly produced in aerobic biological systems, and ·OH, one of the most harmful ROS, is generated from metal-catalyzed interaction of \( \text{O}_2^- \) with \( \text{H}_2\text{O}_2 \). There are three forms of superoxide dismutase (SOD): a cytosolic Cu/Zn-binding homodimeric SOD1, a mitochondrial Mn-dependent homotetrameric SOD2, and an extra-cellular Cu/Zn-binding homotetrameric SOD3 [8, 9]. These SODs efficiently and specifically catalyze the conversion of \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \). Most \( \text{H}_2\text{O}_2 \) and \( \text{LOOH} \) are reduced by glutathione peroxidase (GPX). In mammalian cells, classical GPX (cGPX), plasma GPX (pGPX), intestinal GPX (iGPX) and phospholipid hydroperoxide glutathione peroxidase (PHGPX) have been isolated and functionally characterized [5, 20, 26, 27, 33, 37, 46]. Our study showed that PHGPX could reduce DAG-OOH to DAG-OH, an inactive form of neutrophil stimulation, in the presence of reduced form of glutathione, whereas cGPX and pGPX could not [19]. Furthermore, ebselen, an organic compound possessing PHGPX activity, exhibited a cytoprotective effect on DAG-OOH-induced neuronal cell damage (unpublished data).

VI. Conclusions

In this article, the significance of lipid peroxidation for PKC signaling in physiological and pathological conditions was discussed. Cells and tissues are routinely subjected to some degree of ROS, either exogenously through environmental exposure or endogenously through mitochondrial \( \text{O}_2^- \) leakage and inflammatory processes. Oxidative stress
may occur when the production of ROS including lipid peroxide increases, and/or antioxidative defense or repair system decreases. Various lipid peroxides such as oxidized cholesteryl linoleates, 4-hydroxy-2-nonenal and DAG-OH stimulate PKC molecules and consequently alter the signaling pathway. The non-receptor mediated or unregulated signaling may account for the pathological changes. On the other hand, cellular defense systems against oxidative stress exist in aerobic organisms. PHGPX, which mainly reduces phospholipid hydroperoxide in the cells, can reduce DAG-OH to DAG-OH efficiently. In addition, ebselen, an organic compound possessing PHGPX activity, suppresses DAG-OH induced neuronal cell injury. In summary, alterations of PKC signal transduction induced by lipid peroxides are particularly important for pursuing pathogenesis of oxidative stress.

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VIII. References

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