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Minireview

Role of caspases in TNF-mediated regulation of $cPLA_2$

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Abstract A major part of the proinflammatory activity of tumor necrosis factor (TNF) is brought about by cytosolic phospholipase A_2 ($cPLA_2$) that generates arachidonic acid, the precursor for the production of leukotrienes and prostaglandins. The activation of $cPLA_2$ and induction of proinflammatory lipid mediators is in striking contrast to the teleologic meaning of apoptosis, which is to avoid an inflammatory reaction. In this review we highlight the evidence for a caspase-mediated cleavage and inactivation of $cPLA_2$, which seems to be an important mechanism by which TNF downregulates $cPLA_2$ activity in cells undergoing apoptosis.

Key words: Cytosolic phospholipase A_2; Tumor necrosis factor; Death domain; Caspase

1. Tumor necrosis factor (TNF) and TNF receptors (TNFRs)

TNF represents the prototype of a gene superfamily of ligands that control host defense, inflammation, apoptosis, autoimmunity, and organogenesis [1]. Correspondingly, the receptors for these ligands also constitute a TNFR-related gene superfamily. Most members of the TNF/TNFR family are expressed in the immune system, where they coordinate the protective functions of pathogen-reactive cells. The discovery that cachectin, a protein known to cause fever and wasting, was identical to TNF early on suggested that TNF and its related ligands/receptors are not only protective but can play an important role in human disease. Indeed, anti-TNF antibodies or pharmaceutical inhibitors of TNF have been developed which are now clinically employed to ameliorate inflammatory conditions like rheumatoid arthritis and inflammatory bowel disease that previously proved resistant to conventional antiphlogistic modalities [2].

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Abbreviations: $cPLA_2$, cytosolic phospholipase A_2; DD, death domain; ERK, extracellular signal-regulated kinase; SMase, sphingomyelinase; TNF, tumor necrosis factor; TNFR1, p55 TNF receptor type 1

2. Role of TNF in apoptosis and inflammation

Much is known about how TNF induces programmed cell death (apoptosis) [3]. On the molecular level, apoptosis involves evolutionarily conserved pathways of caspase activation resulting in shrinkage, compaction, and breakdown of the cell into easily phagocytosed membrane-surrounded bits. Purely apoptotic pathways may be involved in tissue remodeling during development or wound repair. It should be emphasized that apoptosis is unable to explain all the death capabilities of TNFRs. TNF also induces cellular necrosis involving swelling or bursting of the cell with organelle degeneration and loss of plasma membrane integrity [4]. In general apoptosis is not found with inflammation. In contrast, necrotic cell death may be extremely important for activating the immune system when TNF-induced necrosis is part of an anti-pathogen response. Thus, the proapoptotic and proinflammatory effects of TNF seem to be mutually exclusive, or at least opposing activities. In the present review, we will propel the idea that one physiological role of the TNF-responsive caspases is to curb the proinflammatory response which might even operate in the absence of apoptotic cell death.

3. TNFR signaling

TNFR-like receptors have unique structural features that couple them directly to signaling pathways controlling cell proliferation, differentiation, activation, and cell death. The hallmark of the TNFR superfamily are pseudorepeats in the extracellular parts typically defined by three intrachain disulfides generated by six highly conserved cysteines [5]. Recent evidence has shown that TNFR self-assemble in the absence of ligand and signaling involves rearrangement of the preassembled chains [6].

TNFR signaling occurs through two principal classes of cytoplasmic adapter proteins: TRAFs (TNFR-associated factors) and 'death domain' (DD) molecules [7]. The signaling adapter is selected depending on whether the cytoplasmic domain of the receptor contains either a TRAF binding motif or a DD. The TRAF binding motif is a stretch of amino acids in the receptor tail that is clutched by a pocket in the globular head group of the adapter through charged residues [1]. TRAF1 and TRAF2 were the first receptor-associated factors identified and shown to interact with the p75 TNFR (TNFR2) [8]. In contrast, the DD is a 60 amino acid globular bundle of six conserved α-helices found in the receptor tail and the adapter that promotes homotypic association [9]. The p55

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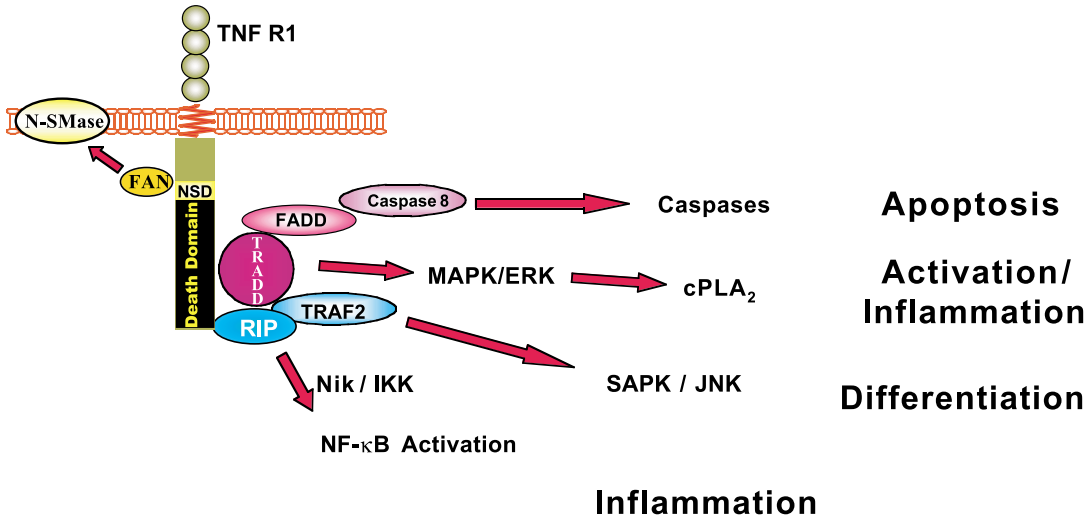

Fig. 1. Signaling pathways emanating from the TNFR1 DD.

TNFR type 1 (TNFR1) through its DD binds the adapter protein TRADD (TNFR-associated DD protein) that itself harbors a DD. TRADD ultimately causes caspase activation and cell death. Since most cellular TNF responses including the activation of cytosolic phospholipase A_2 (cPLA$_2$) are mediated by TNFR1, this review will focus on the signaling of this receptor.

TNFR1 signals rarely result in apoptosis but can trigger several pathways that lead to cell activation, differentiation, or apoptosis (Fig. 1). This is brought about by different adapter proteins that associate with TRADD [7]. At least three proteins can be recruited to TRADD: TRAF2, RIP, and FADD. A large body of evidence indicates that TRAF2 mediates the activation of the c-Jun N-terminal kinase (JNK), RIP is essentially involved in the NF-κB activation pathway, and FADD signals apoptosis through activation of caspase-8. The adapter proteins signaling the phosphorylation and activation of extracellular signal-regulated kinases 1/2 (ERK1/2) have not yet been unambiguously identified. These adapter proteins provide a modularity that allows a regulatory flexibility of TNFR1 signaling events which seems to be required to warrant a specific response among such conflicting signals like cell activation and apoptosis.

4. TNFR1-mediated activation of cPLA$_2$

How TNF signals the activation of cPLA$_2$ is not fully understood. cPLA2 is an 85 kDa protein (Fig. 2) that preferentially liberates arachidonic acid (AA) from the sn-2 position of phospholipid generating lysophospholipid and free AA [19]. AA can be converted to potent inflammatory lipid mediators, the eicosanoids. This conversion occurs enzymatically through the lipoxygenase or cyclooxygenase pathways for the production of leukotrienes, lipoxins, thromboxanes, or prostaglandins. The important role of AA in the regulation of an inflammatory response requires that its levels be tightly controlled. cPLA$_2$ plays a major role in maintaining AA levels, and its enzymatic activity is subject to complex regulatory mechanisms [11]. At least two receptor-mediated events have been identified leading to PLA$_2$ activation: the binding of calcium promotes translocation of cPLA$_2$ to membranes [12], while phosphorylation at Ser505 of cPLA$_2$ directly increases its activity [13]. Activation of cPLA$_2$ occurs in many cell types in response to various stimuli [10]. In particular, cPLA$_2$ can be activated by the death receptors TNFR1 and CD95, which also mediate apoptosis [14]. Thus, two conflicting signals, apoptosis and production of inflammatory medi-

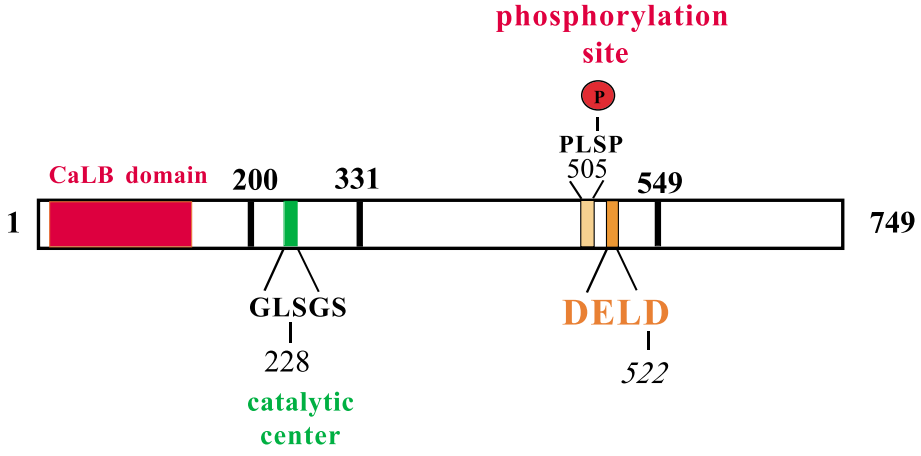

Fig. 2. Schematic representation of the primary structure of cPLA$_2$.

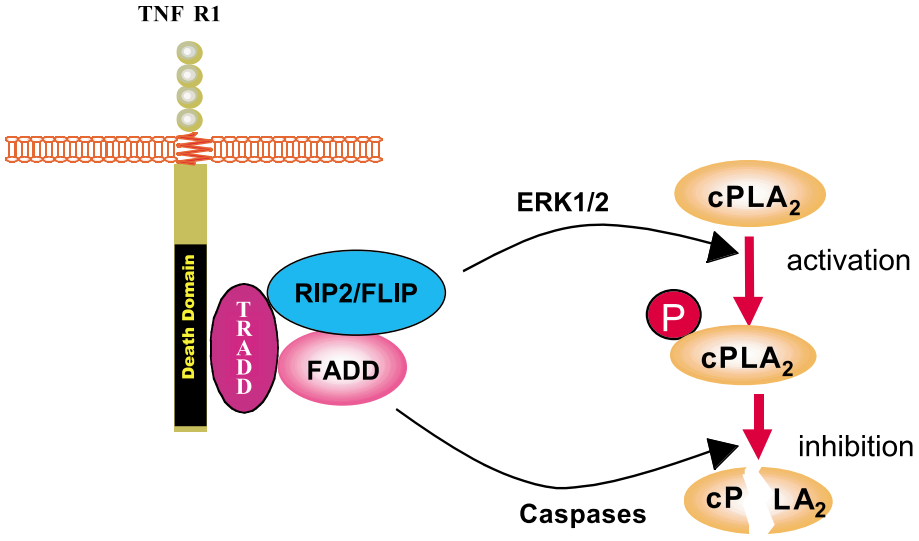

Fig. 3. TNFR1-mediated activation and inactivation of $cPLA_2$.

ators, can emanate from these receptors which, however, are not supposed to coincide.

Based on published evidence from many investigators, a model was proposed where the PLA_2 activation pathway emanating from the TNFR1 involves a ceramide-activated protein kinase (CAPK) that phosphorylates and activates Raf-1 kinase, which eventually results in phosphorylation and activation of ERK1/2 [14]. ERK1/2 in turn have been shown to phosphorylate and thereby activate $cPLA_2$ [13]. It has been suggested that CAPK is identical with KSR, the kinase repressor of Ras [15]. However, the topology and sequence of events leading to the generation of ceramide stimulating the putative CAPK are rather elusive. It was previously proposed that the TNF-responsive, plasma membrane-associated N-sphingomyelinase (N-SMase) provides the CAPK-targeted ceramide [15]. This idea was dismissed, because TNFR1-induced N-SMase activation involves a 'private' signaling pathway that is distinct from that required for PLA_2 activation. TNFR1 signals N-SMase activation through a specific domain termed NSD for N-SMase activating domain [16]. The adapter protein FAN specifically binds to the NSD and mediates activation of N-SMase [17]. By employing two different types of transgenic mice either deficient for FAN or solely lacking an intact TNFR1 DD, we have recently provided genetic evidence that the activation of both ERK and $cPLA_2$ does not require FAN and/or N-SMase but rather is dependent on the presence of a functional DD [18].

The idea that TNF-induced activation of ERK and/or $cPLA_2$ is coupled to the DD of TNFR1 is in agreement with recent observations from other investigators: (i) Boone et al. showed by analyzing TNFR1 mutants stably expressed in L929 cells that TNF induces ERK activation through the DD of TNFR1 [19]. (ii) TNF-induced $cPLA_2$ phosphorylation in human neutrophils was found to be downstream of the TNFR1 DD and to be mediated by p38 MAPK [20]. The activity of p38 MAPK appeared to be regulated by TRAF2 that indirectly interacts with the DD. (iii) RIP2, the receptor-interacting protein 2, has also been shown to mediate TNF-dependent ERK1/2 activation [21]. Since RIP2 belongs to the family of TNFR1 DD binding proteins, this observation also links ERK1/2 activation to the TNFR1 DD. (iv) MADD, a TNFR1-interacting DD protein has been reported as a regulator of TNF-induced ERK1/2 and $cPLA_2$ activation [22]. (v) Finally, Tschopp and his coworkers reported that the FADD binding protein FLIP (FLICE-like inhibitory protein) interacts with the protein kinase Raf-1 resulting in the activation of ERK [23]. While these reports couple various adapter proteins to ERK1/2 and/or $cPLA_2$ activation, these proteins have in common that they bind to the TNFR1 DD. Thus the picture emerges that TNF-induced $cPLA_2$ phosphorylation and activation is triggered through the DD involving the adapter proteins TRADD, FADD and possibly FLIP or RIP2, which results in the activation of protein kinases Raf-1 and ERK1/2 (Figs. 1 and 3).

5. Caspase-mediated inhibition of $cPLA_2$

$cPLA_2$ belongs to the family of numerous substrates that are cleaved by caspases [24–27]. In vitro studies using in vitro translated $cPLA_2$ and recombinant caspases have shown that $cPLA_2$ can be hydrolyzed by caspase-3, -7, and -8. The observed cleavage products of 70 and 32 kDa corresponded to both protein fragments expected after cleavage at the cognate caspase-3 motif DELD present at amino acids 519–522 of the primary $cPLA_2$ structure (Fig. 2). Although the molecular mass of $cPLA_2$ deduced from its amino acid sequence is 85 kDa, the native enzyme migrates on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in the range of 100–110 kDa. The calculated size of the cleavage products would be 59 and 26 kDa, respectively. Since both fragments demonstrate a higher molecular mass on SDS–PAGE, the amino acid sequences responsible for the abnormal migration behavior of $cPLA_2$ are apparently distributed in both cleavage products. When caspases were overexpressed in intact cells, at least five caspases (caspase-1, -3, -4, -7, and -8) produced discrete cleavage patterns of $cPLA_2$. For example, caspase-1 generated mainly a p58 fragment, while caspase-3 and -4 generated mainly p70. Site-specific mutation analysis showed that caspase-3 and -8 cleave $cPLA_2$ at the aspartic acid residue 522, while caspase-1 can cleave human $cPLA_2$ at the YQSD

motif (amino acid residues 456–459) [18,25,26]. It should be noted that overexpression of a single caspase together with $cPLA_2$ likely leads to direct cleavage, but might additionally stimulate the apoptotic program of the cell and, thus, the activation of other caspases that eventually also cleave $cPLA_2$. Thus, the results from cleavage assays in intact cells probably reflect a mixture of fragments generated by direct and indirect processing. It is important to emphasize that the presence of multiple cleavage sites for various caspases assures cleavage of the proinflammatory enzyme $cPLA_2$ in any context leading to apoptosis.

In an earlier report, Wissing et al. showed cleavage of human $cPLA_2$ to a 70 kDa fragment by a caspase-3-like activity [24]. Based on their observation that the tetrapeptide inhibitor of caspase-3 Ac-DEVD-CHO inhibited both TNF-induced apoptosis and release of AA, these investigators suggested that caspase-mediated cleavage of $cPLA_2$ results in the activation of this enzyme. It is, however, highly unlikely that either one of the $cPLA_2$ fragments resulting from the caspase-mediated cleavage at aspartic acid residue 522 (the N-terminal 70 kDa or the C-terminal 32 kDa fragment) can be catalytically active: Previous structure function analysis of $cPLA_2$ revealed residues 200, 228, 331, and 549, located N-terminally as well as C-terminally of the cleavage site at residue 522, to be essential for the catalytic function of $cPLA_2$ [28]. Indeed, $cPLA_2$ activity has not been assayed directly in the Wissing et al. study and the release of AA from apoptotic cells previously labeled with ^{3}H-AA might have been brought about non-specifically [29] or by enzymes other than $cPLA_2$ [30]. In fact, it has been suggested that the PLA_2 isozyme responsible for increased arachidonic acid release during apoptosis may be Ca^{2+}-independent PLA_2 [30].

We and others have recently demonstrated that caspase-mediated cleavage of $cPLA_2$ results, indeed, in the inactivation of its enzymatic activity [25,27]. As predicted from the positions of catalytically essential residues, a recombinant 70 kDa $cPLA_2$ fragment corresponding to the 70 kDa cleavage product had no enzymatic activity, when overexpressed in HEK 293 cells. This indicates that cleavage of $cPLA_2$ at the DELD motif results in inactivation of the enzyme. In addition, mutation of the cognate caspase-3 DELD motif abolished $cPLA_2$ processing in intact cells and lead to increased $cPLA_2$ activity in TNF-stimulated HEK 293 cells. Increased $cPLA_2$ activity after inhibition of caspase-mediated cleavage strongly suggests that caspases downregulate if not terminate $cPLA_2$ activation by hydrolysis into two catalytically dead fragments.

Interestingly, overexpression of the 70 kDa cleavage product of $cPLA_2$ not only showed no detectable enzymatic activity, but exhibited a dominant negative effect on the TNF-induced activation of endogenous $cPLA_2$ [25,30]. A possible explanation might be that the truncated $cPLA_2$ competes for binding of cofactors necessary for the activation process. Such cofactors are calcium ions or proteins phosphorylating $cPLA_2$. Since both the calcium binding domain (CaLB) and the phosphorylation site are still contained in the functionally inert 70 kDa PLA_2 fragment, this overexpressed truncated $cPLA_2$ would efficiently compete for calcium ions or protein kinases. In fact, the catalytically inactive N-terminal fragment was found to be constitutively located at cellular membranes thus potentially inhibiting the stimulus-dependent translocation of native $cPLA_2$ [30]. Therefore, a quantitative cleavage of $cPLA_2$ after stimulation of caspase activity during apoptosis leading to high levels of truncated $cPLA_2$ might downregulate the activity of the remaining intact $cPLA_2$. Indeed, arachidonic acid release after stimulation with calcium ionophore as well as after interleukin-1 treatment is attenuated upon overexpression of the 70 kDa cleavage product of $cPLA_2$. Moreover, the dominant negative N-terminal fragment of $cPLA_2$ was also shown to inhibit arachidonic acid release induced by soluble PLA_2-IIA, but not by soluble PLA_2-X [30]. Taken together, caspase-mediated cleavage of $cPLA_2$ during apoptosis leads to a complete inability of a cell to produce eicosanoids in response to extracellular stimuli.

Many ligands of the TNFR superfamily can induce apoptosis but also activate $cPLA_2$. If both of these actions took place in the same cell, an inflammatory signal would coincide with the apoptotic program that is not meant to lead to an inflammatory response. Thus, the cleavage of $cPLA_2$ by caspases might be an important mechanism that ensures physiologic, programmed cell death to occur in the absence of an inflammatory response.

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References

[1] Locksley, R.M., Killeen, N. and Lenardo, M.J. (2001) Cell 104, 487–501.
[2] Maini,.R.N. and Tayloer, P.C. (2000) Annu. Rev. Med. 51, 207–229.
[3] Ashkenazi, A. and Dixit, V.M. (1998) Science 281, 1304–1308.
[4] Denecker, G., Vercammen, D., Declercq, W. and Vandenabeele, P. (2001) Cell. Mol. Life Sci. 58, 356–370.
[5] Smith, C.A., Farrah, T. and Goodwin, R.G. (1994) Cell 76, 959–962.
[6] Chan, F.K., Chun, H.J., Zhen, L., Siegel, R.M., Bui, K.L. and Lenardo, M.J. (2000) Science 288, 2351–2354.
[7] Wallach, D., Varfolomeev, E.E., Malinin, N.L., Goltsev, Y.V., Kovalenko, A.V. and Boldin, M.P. (1999) Annu. Rev. Immunol. 17, 331–367.
[8] Inuoe, J., Ishida, T., Tsukamoto, N., Kobayashi, N., Naito, A., Azuma, S. and Yamamoto, T. (2000) Exp. Cell Res. 254, 14–24.
[9] Fesik, S.W. (2000) Cell 103, 273–282.
[10] Murakami, M. and Kudo, I. (2002) J. Biochem. 131, 285–292.
[11] Shinohara, H., Balboa, M.A., Johnson, C.A., Balsinde, J. and Dennis, E.A. (1999) J. Biol. Chem. 274, 12263–12268.
[12] Clark, J.D., Lin, L.L., Kriz, R.W., Ramesha, C.S., Sultzman, L.A., Lin, A.Y., Milona, N. and Knopf, J.L. (1991) Cell 65, 1043–1051.
[13] Lin, L.L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A. and Davis, R.J. (1993) Cell 72, 269–278.
[14] Kolesnick, R.N. and Krönke, M. (1998) Annu. Rev. Physiol. 60, 643–665.
[15] Zhang, Y., Yao, B., Delikat, S., Bayoumy, S., Lin, X.-H., Basu, S., McGinley, M., Chan-Hui, P.-Y., Lichenstein, H. and Kolesnick, R. (1997) Cell 89, 63–72.
[16] Adam, D., Wiegmann, K., Adam-Klages, S., Ruff, A. and Krönke, M. (1996) J. Biol. Chem. 271, 14617–14622.
[17] Adam-Klages, S., Adam, D., Wiegmann, K., Struve, S., Kolanus, W., Schneider-Mergener, J. and Krönke, M. (1996) Cell 86, 937–947.
[18] Lüschen, S., Adam, D., Ussat, S., Kreder, D., Schneider-Brachert, W., Krönke, M. and Adam-Klages, S. (2000) Biochem. Biophys. Res. Commun. 274, 506–512.
[19] Boone, E., Vandevoorde, V., de Wilde, G. and Haegeman, G. (1998) FEBS Lett. 441, 275–280.
[20] Waterman, W.H., Molski, T.F.P., Huang, C.-K., Adam, J.L. and Sha'afi, R.I. (1996) Biochem. J. 319, 17–20.
[21] Navas, T.A., Baldwin, D.T. and Stewart, T.A. (1999) J. Biol. Chem. 274, 33684–33690.

[22] Schievella, A.R., Chen, J.H., Graham, J.R. and Lin, L.-L. (1997) J. Biol. Chem. 272, 12069–12075.
[23] Kataoka, T., Budd, R.C., Holler, N., Thome, M., Martinon, F., Irmler, M., Burns, K., Hahne, M., Kennedy, N., Kovacsovics, M. and Tschopp, J. (2000) Curr. Biol. 10, 640–648.
[24] Wissing, D., Mouritzen, H., Egeblad, M., Poirier, G.G. and Jäättelä, M. (1997) Proc. Natl. Acad. Sci. USA 94, 5073–5077.
[25] Adam-Klages, S., Schwandner, R., Lüschen, S., Ussat, S., Kreder, D. and Krönke, M. (1998) J. Immunol. 161, 5687–5694.
[26] Lüschen, S., Ussat, S., Krönke, M. and Adam-Klages, S. (1998) Biochem. Biophys. Res. Commun. 253, 92–98.
[27] Atsumi, G.-I., Tajima, M., Hadano, A., Nakatani, Y., Murakami, M. and Kudo, I. (1998) J. Biol. Chem. 273, 13870–13877.
[28] Pickard, R.T., Chiou, X.G., Strifler, B.A., DeFelippis, M.R., Hyslop, P.A., Tebbe, A.L., Yee, Y.K., Reynolds, L.J., Dennis, E.A., Kramer, R.M. and Sharp, J.D. (1996) J. Biol. Chem. 271, 19225–19231.
[29] Zhang, J., Driscoll, T.A., Hannun, Y.A. and Obeid, L.M. (1998) Biochem. J. 334, 479–485.
[30] Atsumi, G.-I., Murakami, M., Kojima, K., Hadano, A., Tajima, M. and Kudo, I. (2000) J. Biol. Chem. 275, 18248–18258.