Vesicle Membrane Fusion Induced by the Concerted Activities of Sphingomyelinase and Phospholipase C*

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When vesicles composed of an equimolar mixture of sphingomyelin, phosphatidyleholine, phosphatidylethanolamine, and cholesterol are treated with phospholipase C, phospholipid hydrolysis occurs without major changes in vesicle architecture. In the same way, addition of sphingomyelinase leads only to sphingomyelin cleavage. However, when both enzymes are added together, their joint hydrolytic activities give rise to leakage-free vesicle aggregation, lipid mixing, and aqueous contents mixing, i.e. vesicle fusion. The contribution of both enzymes is unequal, the main role of sphingomyelinase being the production of relatively large amounts of ceramide that will facilitate the lamellar-to-nonlamellar transition in the formation of the fusion pore, whereas phospholipase C provides mainly a localized, asymmetric, high concentration of diacylglycerol that constitutes the trigger for the fusion process. The lipidic end-products of both enzymes cooperate in destabilizing and fusing the membranes in a way that is never achieved through the action of any of the enzymes individually, nor by the products themselves when premixed with the other lipids during liposome preparation. Thus the enzymes appear to be coupled through their reaction products. This is the first observation of membrane fusion induced by the concerted activities of two enzymes. Besides, considering that both diacylglycerol and ceramide are important metabolites involved in cell signaling, it may also provide new ideas in the exploration of "cross-talk" phenomena between different signal transduction pathways.

The present view of cell membranes as dynamic structures has put forward the role of membrane fusion in many cellular processes from membrane biogenesis to viral infections. Despite important recent advances, the molecular mechanism of membrane merging leading to fusion of bilayers is not fully understood yet. The so-called "stalk hypothesis" of membrane fusion, first proposed by Kozlov and Markin (1) and then reformulated in slightly different ways by Chernomordik (2) and Siegel (3), constitutes the most widely accepted explanation of this phenomenon at the molecular level. According to this view, fusion would occur through an interbilayer and highly curved semitoroidal intermediate, the stalk, initially formed by the reaction products of the latter study, ceramide is similar to diacylglycerol though contrast, sphingomyelinase yields ceramide, which results in aggregation and leakage of vesicles containing sphingolipid and cholesterol (12). Under certain conditions, sphingomyel- nase can induce leaky fusion of sphingomyelin-containing liposomes (13). Whether physiological fusion events are or are not accompanied by a certain degree of leakage is subject to debate (14). In the present work, we have attempted to combine the specific effects of both enzymes, phospholipase C and sphingo- myelinase, to promote destabilization and fusion of a model membrane system, large unilamellar vesicles, containing both glycerophospholipids and sphingophospholipids.

It is known from our studies on phospholipase C-induced liposomal fusion that diacylglycerol plays two different roles. First, a significant amount (between 5 and 20 mol %) of more or less symmetrically distributed diacylglycerol is required to allow the formation of nonlamellar structures, which are essential for fusion to occur (15–17). This "bulk" diacylglycerol may be included from the start in the liposomal composition, causing a decrease in the lag time between enzyme addition and fusion, although phospholipase C activity is always essential for membrane destabilization (15). The role of phospholipase C consists of generating the pool of diacylglycerol responsible for the second of its two roles: namely the rapid, localized, and asymmetric synthesis of diacylglycerol that is the "trigger" for fusion to occur (15, 16). Under similar conditions, but using vesicles containing sphingomyelin, sphingomyelinase produces aggregation, but not fusion, of liposomes (12). As described in the latter study, ceramide is similar to diacylglycerol though less potent in the induction of nonlamellar phases, and it may substitute for diacylglycerol in reducing the lag time of phospholipase C-induced fusion.

This work shows that, under conditions where neither sphingomyelinase nor phospholipase C produce any major structural change in lipidic vesicles, the concerted action of both enzymes readily induces vesicle fusion, the relatively slow acting sphingomyelinase producing the bulk amount of ceramide and phospholipase C supplying the fast diacylglycerol production that makes the trigger. To our knowledge, this is the first report on

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bilayer fusion induced by the concerted specific actions of two enzymes. In addition, this effect on model membranes may have an in vivo parallelism in what would be a novel aspect of cross-talk between two independent pathways of cell signaling.

MATERIALS AND METHODS

Phospholipase C (EC 3.1.4.3) from Bacillus cereus was supplied by Boehringer Mannheim. Cholesterol and sphingomyelin (EC 3.1.4.12) from B. cereus were from Sigma. Sphingomyelinase contained an impurity of phospholipase C, in a proportion that varied from batch to batch. When the specific activity of phospholipase C was \( \leq 2\% \) of that of sphingomyelinase, the enzyme was used as such. Batches with contamination higher than 10% were discarded. In intermediate situations, sphingomyelinase was used in experiments when no phospholipase C was involved, in the presence of 2 mM \( \alpha \)-phenanthroline. Preliminary studies showed that the presence of the phospholipase C inhibitor \( \alpha \)-phenanthroline did not affect sphingomyelinase activity, in agreement with published data (18).

Addition of phospholipase C to a suspension of large unilamellar vesicles consisting of PC:SM:PE:Ch (1:1:1:1, mole ratio) leads to rapid liposomal aggregation and fusion (Nieva et al., 1989). However, when PC is substituted by an equimolar mixture of PC and SM, so that the new liposomal composition is PC:SM:PE:Ch (1:1:1:1), addition of phospholipase C, under otherwise similar conditions, produces neither fusion (contents mixing) nor lipid mixing (Fig. 1A) even in a time scale that is 1 order of magnitude longer than the one used in the previous study. Light scattering does not change either in the experiment shown in Fig. 1A, which precludes vesicle aggregation under those conditions (Fig. 2). However, lipid hydrolysis occurs, and the amount of diacylglycerol produced in the experiment shown in Fig. 1A should be enough to produce vesicle fusion. At least in the PC:PE:Ch mixture, enzymically produced diacylglycerol in amounts equivalent to 5–20% of the total lipid was found to induce fusion (15).

Similarly, vesicles containing SM:PE:Ch (2:1:1) undergo rapid aggregation and leakage, but no lipid or contents mixing, when treated with sphingomyelinase (12). The same enzyme treatment on large unilamellar vesicles consisting of PC:SM:PE:Ch (1:1:1:1) produces lipid hydrolysis, but no aggregation or fusion (Figs. 1B and 2). Remarkably, however, the joint addition of both phospholipase C and sphingomyelinase to PC:SM:PE:Ch (1:1:1:1) vesicles leads to rapid vesicle aggregation, lipid mixing, and contents mixing (Figs. 1C and 2). When both enzymes are added together, their activities appear to be mutually potentiated, and phospholipase C becomes particularly activated (Fig. 1D). None of the effects shown in Figs. 1, C and D, are seen when the enzymes had been previously inactivated by heat, or when phospholipase C had been preincubated with its specific inhibitor, \( \alpha \)-phenanthroline. In the experiments in Fig. 1, C and D, 0.4 units/ml each phospholipase C and sphingomyelinase were added. Qualitatively similar results were found when the amounts of each enzyme varied in the 0.4–1.6 units/ml range, both enzymes being present at
equal concentrations, only the rates increased with increasing amounts of enzyme.

Fusion induced by phospholipase C occurred in the absence of vesicle leakage (10), whereas sphingomyelinase action on SM:PE:Ch vesicles included extensive release of aqueous contents (12). With PC:SM:PE:Ch (1:1:1:1) liposomes, neither enzyme produces vesicle leakage (Fig. 3) although, when both enzymes are added in combination, a leakage effect is observed that starts once fusion (mixing of aqueous contents) has reached an apparent equilibrium (Fig. 3). Under these conditions leakage proceeds slowly for a long time afterward.

The behavior of the two enzymes can be explained by assuming that, in the experiments described in Fig. 1, (a) the rate of glycerophospholipid hydrolysis by phospholipase C acting on PC:SM:PE:Ch (1:1:1:1) vesicles (Fig. 1A) is too slow to permit the buildup of a localized, asymmetric pool of diacylglycerol that may act as a fusion trigger (15, 16). A local high concentration of diacylglycerol in one of the monolayers can only be built up in competition with the phenomena of lateral and transbilayer (flip-flop) diffusion. (b) Sphingomyelinase acts in much the same way as in the ternary mixture SM:PE:Ch (1:1:1) (12), and consequently no fusion occurs. However, a significant proportion of ceramide is generated in the bilayers, thus facilitating an eventual lamellar transition. (c) When both enzymes are acting together, the rate of generation of ceramide and particularly of diacylglycerol is high enough to overcome diffusion, and vesicle fusion occurs (Fig. 1, C and D). These points are discussed and experimentally tested below.

The initial rates of phospholipase C and of sphingomyelinase acting on 3- and 4-component liposomes are shown in Table I. In these cases, initial rates are also maximum rates since no “lag periods” are detected. The amounts of end product (diacylglycerol or ceramide) that accumulated after 1 min of enzyme action are also lower in the 4-component than in the 3-component systems. It should be noted that, with PC:SM:PE:Ch vesicles, sphingomyelinase does not elicit vesicle aggregation (Fig. 2), as was the case with SM:PE:Ch vesicles (12). However, independent studies have shown that vesicle aggregation induced by sphingomyelinase is extremely sensitive to lipid composition.2

When both enzymes are added together to the 4-component system, the amount of substrate processed after 1 min is approximately three times the sum of the amounts of substrate hydrolyzed by each single enzyme (Table I). This is in agreement with the mutual potentiation of both enzymes when acting on this system, which was as seen in Fig. 1D. The data in Table I and Fig. 1D are compatible with the hypothesis that it is the faster rate of diacylglycerol production that leads to fusion under the conditions of Fig. 1C, as compared with those of Fig. 1A, because, under those conditions, fast diacylglycerol synthesis may give rise to a steady state in which local accumulation of this product occurs. It is doubtful that the concomitant activation of sphingomyelinase is instrumental in the onset of fusion because, in the previous study (12), it was shown that fast ceramide production leads to vesicle leakage, but not fusion. However, full experimental confirmation of this point will require specific measurements of diacylglycerol and ceramide diffusion in bilayers.

The hypothesis that, in the fusion process shown in Fig. 1C, each enzyme plays a specific role so that sphingomyelinase produces ceramide that mainly facilitates the formation of non-lamellar intermediates, while phospholipase C provides the trigger, leads to the prediction that the order of addition of the enzymes should not be irrelevant. Sphingomyelinase would need some time to build up an adequate concentration of ceramide, whereas the action of phospholipase C should be much faster in time. Consequently, when both enzymes are added simultaneously, or when phospholipase C is added prior to sphingomyelinase, a lag time should be seen, corresponding to the synthesis of ceramide by the latter enzyme.

2 M. B. Ruiz-Argüello, unpublished results.
addition of sphingomyelinase first, followed after some time by phospholipase C, should lead to immediate fusion. These predictions are confirmed by the experiments in Fig. 4. Changes in light scattering parallel the observations on lipid and contents mixing, with a lag time when phospholipase C is added prior to sphingomyelinase, but not vice versa (data not shown).

A related prediction of our hypothesis is that, if the liposomal composition contains a sufficient amount of ceramide from the start, sphingomyelinase would become "redundant," and phospholipase C alone would be able to induce fusion. This was tested by preparing large unilamellar vesicles with the usual 4-component mixture, in which 15% sphingomyelin was substituted by ceramide so that the final bilayer composition was PC:SM:PE:Ch:ceramide (25:10:25:25:15 mole ratio). These vesicles were stable for several days at 4 °C. Addition of phospholipase C immediately produced vesicle fusion (Fig. 5), thus reinforcing our hypothesis. Under these conditions, fusion is slower than when both enzymes are added (compare Figs. 5 and 1C). This may be because of the transbilayer distribution of enzymically produced ceramide being partially asymmetric, which should favor the fusion process (3), whereas ceramide mixed with the other lipids in the process of vesicle preparation will be symmetrically distributed in the bilayers. Note that, for the first 5 min, the presence of ceramide in the bilayer does not modify significantly the production of diacylglycerol by phospholipase C (Fig. 5B), and yet fusion occurs because, once ceramide is present in sufficient amounts to facilitate nonlamellar phase formation, a small, but localized, formation of diacylglycerol by the enzyme is enough to trigger fusion. Conversely, vesicles prepared with a PC:SM:PE:Ch:diacylglycerol (17.5:25:17.5:25:15) mixture did not fuse when treated with sphingomyelinase (Fig. 5A), in agreement with the proposed role of phospholipase C activity in the induction of fusion.

The requirement of a fast generation of diacylglycerol to act as a trigger for vesicle fusion was further tested experimentally with the PC:PE:Ch (2:1:1) + phospholipase C system. A series of experiments were carried out in which initial rates of enzyme activity and of liposome fusion (contents mixing) were measured, in the presence of increasing concentrations of a specific inhibitor of phospholipase C, o-phenanthroline. The results are shown in Fig. 6. As expected, o-phenanthroline inhibits the phosphohydrolase activity, and consequently, fusion is inhibited. The interesting point, however, is that once the enzyme activity goes below ~25% of the native value, fusion becomes zero. This occurs at about 0.15 mM inhibitor, whereas enzyme activity is stopped only with 10-fold concentrations of o-phenanthroline. Thus a threshold rate of diacylglycerol production is required for fusion to occur. This result had been anticipated by the observations of Nieva et al. (10) showing that, below a certain enzyme/vesicle ratio, fusion of vesicles did not take place.

TABLE I

Enzyme activities on phospholipid substrates in the form of large unilamellar vesicles

| Lipid composition | Enzyme | Initial rate | Product accumulated after 1 min | Product accumulated after 1 min |
|-------------------|--------|--------------|---------------------------------|---------------------------------|
|                   |        | nmoles/min   | %                               | nmoles                           |
| PC:PE:Ch (2:1:1)  | Phospholipase C | 65.4 ± 0.24 | 24.3 ± 1.94                   | 65.6 ± 0.26                      |
| SM:PE:Ch (2:1:1)  | Sphingomyelinase | 35.2 ± 1.06 | 12.8 ± 0.17                   | 38.3 ± 0.35                      |
| PC:SM:PE:Ch (1:1:1:1) | Phospholipase C | 24.0 ± 1.62 | 15.2 ± 0.86                   | 20.5 ± 1.04                      |
| PC:SM:PE:Ch (1:1:1:1) | Sphingomyelinase | 14.4 ± 0.85 | 11.0 ± 1.03                   | 9.2 ± 1.12                       |
| PC:SM:PE:Ch (1:1:1:1) | Both | ND | 30.7 ± 1.85 | 92.1 ± 5.55 |

a Enzyme concentration is always 1.6 units/ml. When both enzymes are added simultaneously, 0.4 units/ml of each are added.

b 100% corresponds to the total lipid in the mixture, either hydrolyzable or not.

c The total amount of lipid is 300 nmol.

**DISCUSSION**

The above results show that the combined actions of two enzymes, sphingomyelinase and phospholipase C, can promote the aggregation, lipid mixing, and contents mixing of large unilamellar vesicles consisting of equimolar amounts of SM, PC, PE, and Ch. The process is leakage-free, although some leakage occurs once the rapid fusion event has taken place. Considered together, these observations can be interpreted in terms of enzyme-induced vesicle fusion, representing a model system for cell membrane fusion. This may be of interest because the molecular mechanisms involved in the merging of lipid bilayers in biomembrane fusion are not understood fully yet.

The novelty of our system consists of the involvement of two enzymes, each with a specific role (Fig. 1). Results from this laboratory showed for the first time fusion of liposomes induced by the catalytic action of an enzyme, namely phospholipase C (10). It should be noted that in our system a fusion occurs through the catalytic action of both enzymes. Hydrolytically inactive enzymes are also inactive in promoting fusion. One of the enzymes, sphingomyelinase, can be omitted if the bilayer contains a sufficient amount of ceramide, but even then phospholipase C induces only a relatively slow fusion (Fig. 5). We demonstrate here that both enzymes have to be present for rapid fusion to occur, and that the order of enzyme addition is...
relevant to the final outcome (Fig. 4). Thus, we are dealing with an effect resulting from the concerted activities of both enzymes. To the authors’ knowledge, an equivalent phenomenon has never been shown previously in a vesicle system, although Fanani and Maggio (24) have recently described the mutual modulation of sphingomyelinase and phospholipase A2 activities in mixed lipid monolayers. The results described in this paper represent a step forward in the understanding of the complex cell membrane fusion systems that occur as the final result of numerous enzymic events (25).

Central to the understanding of our 2-enzyme fusion system is the steady-state equilibrium between the generation of membrane-destabilizing lipids (diacylglycerol, ceramide) in the bilayer and their lateral and transmembrane diffusion. Numerous lines of evidence from this (Figs. 4–6 and Table I) and other studies (10, 15, 16) point toward the requirement of a localized, asymmetric generation of fusogenic lipid for the onset of fusion. Such a "hot spot" can only arise if the synthesis of diacylglycerol, and to a smaller extent ceramide, is fast enough to overcome the diffusion of these lipids along the bilayer. This is an example of what has been called “surface dilution kinetics” (26, 27).

From the point of view of membrane fusion, the current views of the underlying molecular mechanism support the so-called stalk hypothesis, first proposed by Kozlov and Markin (1) and then subsequently reexposed by Siegel (3) and Chernomordik (2). The stalk is a highly bent lipidic structure whose formation would be facilitated by lipids inducing a negative curvature in their respective monolayers, e.g. diacylglycerol or ceramide. The stalk would then evolve toward the formation of a fusion pore. There is also experimental evidence suggesting that the fusion pore may be lipidic in structure (28, 29). In our previous studies, the relationship between the stalk and the structure of nonlamellar, particularly inverted cubic phases has been put forward (16, 30). In our view, the stalk would represent a localized structure whose geometry would correspond to that of an inverted cubic phase (4). The results in this paper support the stalk-pore model. In particular, the prediction that not only the presence of lipids inducing a negative curvature but also their asymmetric distribution will enhance stalk formation and fusion is fulfilled by the experimental observations above.

It has often been said that meaningful models of cell membrane fusion should be nonleaky. This has recently been questioned by data showing that influenza virus fusion is a very leaky process (14, 31). Under certain conditions, we have also found a very leaky liposome fusion process induced by sphingomyelinase (13). But there are eukaryotic fusion processes that must be nonleaky, e.g. secretion. In our case (Fig. 3), the fusion process itself is nonleaky although the end of the rapid fusion stage is followed by one of steady, slow leakage. The release of aqueous contents is probably, in our case, peripheral to the fusion event and perhaps related to the extensive leakage caused by sphingomyelinase when acting on SM:PE:Ch liposomes (12, 13).

The physiological relevance of the above observations may go beyond their relationship to cell membrane fusion events because diacylglycerol and ceramides are prominent members of two important families of lipid second messengers (8, 9, 32–34). In particular, the interaction or “cross-talk” between the glycerophospholipid- and sphingolipid-derived signaling pathways has been shown to be very important for metabolic regulation (35). Many aspects of this cross-talk are still unclear. Ceramide and diacylglycerol have been shown to elicit opposing effects in many cell types. Whereas ceramide can induce programmed cell death (apoptosis) (33), diacylglycerol is anti-apoptotic (36). However, it has been demonstrated that sphingomyelin, or its metabolite ceramide, can effectively potentiate the growth factor-stimulated proliferation of Swiss 3T3 fibroblasts (37). Growth factors are known to induce diacylglycerol generation through activation of different PLC isoforms, or via the coordinated action of phospholipase D and phosphatidate phosphohydrolase (35). Interestingly, although ceramide elevation induces apoptosis, when it is combined with an elevation of diacylglycerol, cell proliferation prevails over cell death (36). Therefore, the synergistic effect of ceramide on growth factor-induced cell proliferation might be mediated, at least in part, by an interaction of ceramide with diacylglycerol. The data presented here support this hypothesis. Whereas treatment of vesicles with phospholipase C or sphingomyelinase to generate diacylglycerol or ceramide, respectively, has no significant effect on vesicle architecture, the combined action of both enzymes leads to vesicle fusion. The concentrations of diacylglycerol and ceramide generated in our in vitro system are relatively high compared with the concentrations that can occur intracellularly. However, it is possible that high concentra-
tions of diacylglycerol and ceramide can be generated locally in vivo. This might cause changes in specific domains of biological membranes, and as a consequence, it could modify important signal transduction events. Because membrane fusion plays an obvious role in membrane biogenesis and control of eukaryotic metabolism, these novel observations may help in exploring hitherto unknown interactions between lipid-mediated cell signaling pathways.

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REFERENCES

1. Kozlov, M. M., and Markin, V. S. (1983) Biofizika 28, 255–261
2. Chernomordik, L. V. (1996) Chem. Phys. Lipids 81, 203–213
3. Siegel, D. P. (1993) Biophys. J. 65, 2124–2140
4. Luzzati, V. (1997) Curr. Opin. Struct. Biol. 7, 661–668
5. Chernomordik, L. V., Chanturiya, A., Green, J., and Zimmerberg, J. (1995) Biophys. J. 69, 922–929
6. Chernomordik, L. V., Leikina, E., Frolov, V., Bronk, P., and Zimmerberg, J. (1997) J. Cell Biol. 136, 81–93
7. Siegel, D. P., and Epand, R. M. (1997) Biophys. J. 73, 3089–3111
8. Berridge, M. J. (1987) Annu. Rev. Biochem. 56, 159–193
9. Obeid, L. M., and Hannun, Y. A. (1995) J. Cell. Biochem. 58, 191–198
10. Nieva, J. L., Goti, F. M., and Alonso, A. (1989) Biochemistry 28, 7364–7367
11. Goñi, F. M., Nieva, J. L., Basáñez, G., Fidelio, G. D., and Alonso, A. (1994) Biochim. Biophys. Acta 329, 839–844
12. Ruiz-Argüello, M. B., Basáñez, G., Goti, F. M., and Alonso, A. (1996) J. Biol. Chem. 271, 26616–26621
13. Basáñez, G., Ruiz-Argüello, M. B., Alonso, A., Goñi, F. M., Karlsson, G., and Edwards, K. (1997) Biophys. J. 72, 2630–2637
14. Shangguan, T., Alford, D., and Bentz, J. (1996) Biochemistry 35, 4956–4965
15. Nieva, J. L., Goti, F. M., and Alonso, A. (1995) Biochemistry 34, 1054–1058
16. Nieva, J. L., Basáñez, G., Goñi, F. M., Gulik, A., Vargas, R., and Luzzati, V. (1995) FEBS Lett. 368, 143–147
17. Basáñez, G., Nieva, J. L., Rivas, E., Alonso, A., and Goñi, F. M. (1996) Biophys. J. 70, 2299–2306
18. Ikezawa, H., Mori, M., Ohyabu, T., and Taguchi, R. (1978) Biochim. Biophys. Acta 528, 247–256
19. Mayer, L. D., Hope, M. H., and Cullis, P. R. (1986) Biochim. Biophys. Acta 858, 161–168
20. Böttcher, C. S. F., van Gent, C. M., and Fries, C. (1961) Anal. Chim. Acta 61, 297–303
21. Hoekstra, D., de Boer, T., Klappe, K., and Wilschut, J. (1984) Biochemistry 23, 5675–5681
22. Ellens, H., Bentz, J., and Szeka, F. C. (1986) Biochemistry 25, 4141–4147
23. Hamilton, J. A., Bhamidipati, S. P., Kodali, D. R., and Small, D. M. (1991) J. Biol. Chem. 266, 1177–1186
24. Fanani, M. L., and Maggio, B. (1997) Mol. Membr. Biol. 14, 25–29
25. Mallabiarabrena, A., and Malhotra, V. (1995) Cell 83, 667–669
26. Dennis, E. A. (1973) Arch. Biochem. Biophys. 158, 485–493
27. Carman, G. M., Deems, R. A., and Dennis, E. A. (1995) J. Biol. Chem. 270, 18711–18714
28. Monck, J. R., and Fernandez, J. (1994) Neuron 12, 707–716
29. Zimmerberg, J., Vogel, S. S., and Chernomordik, L. V. (1993) Annu. Rev. Biophys. Biomol. Struct. 22, 433–466
30. Basáñez, G., Goñi, F. M., and Alonso, A. (1998) Biochemistry 37, 3901–3908
31. Gunther-Aushorn, S., Pratzer, A., and Stegmann, T. (1995) J. Biol. Chem. 270, 29279–29285
32. Nishizuka, Y. (1995) FASEB J. 9, 484–496
33. Hannun, Y. A. (1996) Science 274, 1855–1859
34. Ghosh, S., Strum, J. C., and Bell, R. M. (1997) FASEB J. 11, 45–50
35. Gómez-Muñoz, A., Aboulsalham, A., Kikutchi, Y., Waggoner, D., and Brindley, D. N. (1997) in Sphingolipid-Mediated Signal Transduction (Hannun, Y. A., ed), pp. 103–120, RG Landes Co., NY
36. Spiegel, S., Foster, D., and Koelen, R. (1996) Curr. Opin. Cell Biol. 8, 150–167
37. Sasaki, T., Hazeki, K., Hazeki, O., Ut, M., and Katada, T. (1995) Biochem. J. 311, 829–834