Activation, Inhibition, and Regiospecificity of the Lysophospholipase Activity of the 85-kDa Group IV Cytosolic Phospholipase A₂

Richard W. Loo, Kilian Conde-Frieboes, Laure J. Reynolds, and Edward A. Dennis†‡

From the Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California 92093-0601

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 272, No. 31, Issue of August 1, pp. 19214–19219, 1997

© 1997 by The American Society for Biochemistry and Molecular Biology, Inc.

Fax: 619-534-7390; E-mail: edennis@ucsd.edu.

Activation, Inhibition, and Regiospecificity of the Lysophospholipase Activity of the 85-kDa Group IV Cytosolic Phospholipase A₂

The 85-kDa Group IV calcium-dependent cytosolic phospholipase A₂ (cPLA₂) catalyzes the hydrolysis of palmitoylglycero-3-phosphocholine to palmitic acid and glycero-3-phosphocholine. Palmitoylglycero-3-phosphocholine exists as a 9:1 equilibrium mixture of the sn-1 and sn-2 isomers, with the fatty acid predominately at the sn-1 position. We have monitored this reaction by 31P NMR to determine which palmitoylglycero-3-phosphocholine isomer is processed by cPLA₂. When both lysophospholipid isomers are present in a 1:1 mixture under conditions in which acyl migration is minimized, cPLA₂ rapidly consumes both isomers. However, 1-palmitoylglycero-3-phosphocholine is consumed seven times faster than the 2-palmitoylglycero-3-phosphocholine isomer. We have previously reported that this lysophospholipase reaction is accelerated in the presence of glyceral. We now find that this apparent increase in activity is accounted for, in part, by glyceral acting as an alternative acceptor for the cleaved fatty acid, as is the case for this enzyme’s phospholipase A₂ (PLA₂) activity. In contrast, dioleoylglycerol, which accelerates the PLA₂ activity, does not act as an acceptor in either the lysophospholipase or the PLA₂ reaction, but can affect enzyme activities by altering substrate presentation. We also show that a known inhibitor of the PLA₂ activity of cPLA₂ is able to inhibit its lysophospholipase activity with a similar IC₅₀ to its PLA₂ activity. However, the effect of inhibitors is dependent on the manner in which they are presented to the enzyme.

Phospholipase A₂ (PLA₂)¹ comprises a family of enzymes that catalyze the hydrolysis of fatty acids from the sn-2 position of phospholipids (1). The Group IV calcium-dependent cytosolic phospholipase A₂ (cPLA₂) is an 85-kDa member of this family that displays a preference for phospholipids that contain arachidonic acid (2–7). Since arachidonic acid is a second messenger for a number of cellular functions and is also a precursor of various modes of activation and inhibition of the two activities.
**Lysophospholipase Activity of cPLA<sub>2</sub>**

**EXPERIMENTAL PROCEDURES**

*Materials—<sup>1</sup>4C*Palmitoylglycero-3-phosphocholine and <sup>1</sup>4C*arachidonoylglycero-3-phosphocholine were purchased from NEN Life Science Products. 1-<sup>14</sup>CPalmitoyl-2-[14C]palmityl-2-<sup>14</sup>C]palmitoylglycero-3-phosphocholine was purchased from Amersham Corp. Nonradioactive lipids were purchased from Avanti Polar Lipids. All-cis,5,8,11,14-nonadecatetraenyl trifluoromethyl ketone (arachidonyl trifluoromethyl ketone (AA-TFKK)) (18) and 2,3-dioxoocetadecanoic acid tert-butyl ester monohydrate (palmityltricarbonyl (PA-TC)) (19) were purchased as described elsewhere. Rhizopus arrhizus lipase was purchased from Boehringer Mannheim. Deuterated solvents were from Cambridge Isotopes. Recombinant cPLA<sub>2</sub> was generously provided by Dr. Ruth Kramer (Lilly Research Laboratories).

2-Palmitoylglycero-3-phosphocholine (2-PGPC) was prepared by treating 1,2-dipalmitoyl-sn-glycero-3-phosphocholine with Rhizopus lipase following published procedures (20). Conversion of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine to 2-PGPC did not proceed to completion. However, palmitoylglycero-3-phosphocholine could be separated from a mixture with the starting material by chromatography on Sephadex LH-20. 2-PGPC prepared in this manner gave a single spot by analytical TLC, but showed two peaks by <sup>31</sup>P NMR corresponding to a 4:1 mixture of palmitoylglycero-3-phosphocholine isomers (the minor isomer being 1-PGPC). Similarly, commercial 1-PGPC contains ~10% of the corresponding 2-PGPC isomer. These cross-contaminations have been documented and are attributed to migration of the fatty acyl chain during the preparation and purification of lysophospholipids (20).

**<sup>31</sup>P NMR Assay**—Samples were made up in a 35:35:30 (v/v/v) mixture of 200 mM Hepes (pH 7, with 10 mM CaCl<sub>2</sub> and 150 mM NaCl), D<sub>2</sub>O, and <sup>5</sup>mL preparation and purification of lysophospholipids (20).

**PLA<sub>2</sub> Mixed Micelle Assay—**Assays were performed in a standard buffer composed of 80 mM Hepes (pH 7.4), 150 mM NaCl, 10 mM CaCl<sub>2</sub>, 1 mg/ml bovine serum albumin, and 1 mM dithiothreitol. The mixed micelle assay also contained 1 mM 1-palmitoyl-2-arachidonoylglycero-3-phosphocholine (PAPC) (with 100,000 cpm [<sup>14</sup>C]PAPC), 2 mM Triton X-100, 30% glycerol, and 3.75 μg/ml cPLA<sub>2</sub> in a volume of 200 μL. The substrate solution was prepared as described previously (19). Assays were run for 40 min at 40 °C. The reaction was quenched and worked up also as described previously (19).

**Lysophospholipase Mixed Micelle Assay—**The lysophospholipase activity was measured in a mixed micelle assay that contained 1 mM commercial 1-palmitoylglycero-3-phosphocholine (lyso-PC)<sup>2</sup> (with 100,000 cpm lyso-[<sup>14</sup>C]PC), 2 mM Triton X-100, 30% glycerol, and 0.5 μg/ml cPLA<sub>2</sub> in 200 μL of the standard buffer described above. The substrate was vortexed to clarity instead of probe sonicating. Assays were also run for 40 min at 40 °C and quenched using the modified Dole procedure (21).

**Dual-substrate Assay—**Assays containing both PAPC and lyso-PC in mixed micelles were composed of 1 mM PAPC, 1 mM lyso-PC (with 100,000 cpm either [<sup>14</sup>C]PAPC or lyso-[<sup>14</sup>C]PC), 4 mM Triton X-100, 30% glycerol, and 3.75 μg/ml cPLA<sub>2</sub> in 200 μL of the standard buffer. Assays were prepared and run analogous to the PC mixed micelle assay described above. Those assays containing [<sup>14</sup>C]PAPC were worked up as described for the PLA<sub>2</sub> mixed micelle assay, whereas those with lyso-[<sup>14</sup>C]PC were worked up as described for the lysophospholipase mixed micelle assay.

**PC/DAG Assay—**The PC/DAG assay was similar to that described by Kramer et al. (4), but utilized a higher concentration of substrate. This assay contained 20 μM PAPC (with 100,000 cpm [<sup>14</sup>C]PAPC), 10 μM 1,2-dioleoylglycerol, and 15–25 ng/ml cPLA<sub>2</sub> in 200 μL of the standard buffer. The substrate was prepared by adding 3 × assay buffer to the dried PAPC/DAG and probe sonicating. Assays were run for 15 min at 40 °C and extracted using the Dole procedure as described above for the PLA<sub>2</sub> mixed micelle assay.

**TLC Assay—**Assays that required separation of fatty acid and mono- or triglyceride products utilized the same substrate, buffer components, and assay conditions as described above. The reactions were quenched with 500 μL of chloroform/methanol/acetic acid (2:4:1, v/v/v) instead of Dole reagent and then vortexed. To this, 1.0 mL of water and 500 μL of chloroform were added, and the mixture was vortexed again. This solution was then centrifuged (1000 × g for 2 min) to separate the organic and aqueous layers. 500 μL of the organic layer was transferred to another test tube, and the solvent was evaporated in a vacuum oven overnight at 40 °C. The resulting lipid film was resuspended in 50 μL of chloroform/methanol (2:1, v/v) and loaded onto TLC prep plates. Unlabeled fatty acid, monoglyceride, and/or triglyceride standards were added to the prep plate. The TLC plate was then developed in hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The resulting TLC plate was visualized with iodine, and zones corresponding to fatty acid, starting phospholipid, monoglyceride, and/or triglyceride were scraped and counted. Blanks were conducted for every data point and subtracted from the corresponding data point.

**RESULTS**

**Regiospecificity of the Lysophospholipase Activity of cPLA<sub>2</sub>**—Under the experimental conditions described, 1-PGPC, 2-PGPC, and GPC displayed distinct <sup>31</sup>P NMR signals (Table I). This permitted simultaneous in situ monitoring of the three species by <sup>31</sup>P NMR. Fig. 2 shows the effect of cPLA<sub>2</sub> on an equimolar mixture of the two lysophospholipid isomers. In the presence of 2-Palmitoylglycero-3-phosphocholine used in the radiochemical assays was a mixture of sn-1 and sn-2 regioisomers and is generally referred to as lyso-PC in the text.
cPLA₂, both palmitoylglycero-3-phosphocholine isomers were rapidly consumed (<20% of the total starting palmitoylglycero-3-phosphocholine remained after 2 h). In addition, the consumption of palmitoylglycero-3-phosphocholine coincided with the expected appearance of GPC (Fig. 3A). In the absence of cPLA₂, no GPC formation was observed even after 24 h.

The time courses for the disappearance of both palmitoylglycero-3-phosphocholine isomers can be fit to first-order rates of decay. The hydrolysis of 1-PGPC displays an apparent first-order rate constant of 0.087 ± 0.008 min⁻¹, whereas the hydrolysis of 2-PGPC has an apparent first-order rate constant of 0.013 ± 0.001 min⁻¹. Thus, in the presence of cPLA₂, 1-PGPC is consumed seven times faster than its 2-PGPC isomer.

By comparison, the formation of GPC is not a simple first-order exponential process. Instead, it appears to be composed of a combination of two first-order processes (Fig. 3B). There is an initial fast formation of GPC, followed by a slower rate of GPC production. This result is consistent with a picture of GPC formation was observed even after 24 h.

The time courses for the disappearance of both palmitoylglycero-3-phosphocholine isomers can be fit to first-order rates of decay. The hydrolysis of 1-PGPC displays an apparent first-order rate constant of 0.087 ± 0.008 min⁻¹, whereas the hydrolysis of 2-PGPC has an apparent first-order rate constant of 0.013 ± 0.001 min⁻¹. Thus, in the presence of cPLA₂, 1-PGPC is consumed seven times faster than its 2-PGPC isomer.

By comparison, the formation of GPC is not a simple first-order exponential process. Instead, it appears to be composed of a combination of two first-order processes (Fig. 3B). There is an initial fast formation of GPC, followed by a slower rate of GPC production. This result is consistent with a picture of GPC formation was observed even after 24 h.

The time courses for the disappearance of both palmitoylglycero-3-phosphocholine isomers can be fit to first-order rates of decay. The hydrolysis of 1-PGPC displays an apparent first-order rate constant of 0.087 ± 0.008 min⁻¹, whereas the hydrolysis of 2-PGPC has an apparent first-order rate constant of 0.013 ± 0.001 min⁻¹. Thus, in the presence of cPLA₂, 1-PGPC is consumed seven times faster than its 2-PGPC isomer.
ing both lyso-PC and PAPC substrates. In contrast, DAG does activate both the PLA₂ and lysophospholipase activities when Triton X-100 is not present (data not shown).

**Inhibition of the Lysophospholipase Activity of cPLA₂**—The ability of activated ketones to inhibit the lysophospholipase activity of cPLA₂ was evaluated. Fig. 6A shows the effect of AA-TFMK, PA-TC, and anandamide on the lysophospholipase activity. The activity of the cPLA₂ control in this assay was 1.5 μmol/min/mg, higher than the activity observed in a corresponding PLA₂ assay (0.2–0.7 μmol/min/mg) (19). This higher activity using a lyso-PC substrate has been noted previously (10).

In this assay, AA-TFMK inhibited the lysophospholipase activity of cPLA₂ with an IC₅₀ of 70 μM (0.023 mol fraction). PA-TC and anandamide did not inhibit the enzyme. This is in contrast to an assay of the enzyme’s PLA₂ activity with a PAPC substrate, where AA-TFMK (19), PA-TC (19), and anandamide all appeared to inhibit the enzyme. Instead, in this assay, both PA-TC and anandamide caused an increase in the lysophospholipase activity.

To clarify the observed differences in inhibition by these compounds of the PLA₂ and lysophospholipase activities of cPLA₂, activity was also examined under a different PLA₂ assay condition. In the PC/DAG assay, AA-TFMK inhibited enzyme activity with an IC₅₀ of 0.65 μM (0.021 mol fraction) (Fig. 6B), whereas the presence of PA-TC and anandamide had no effect in this assay.

In a final experiment, we assayed both activities under identical assay conditions. We ensured that the PAPC and lyso-PC substrates were presented in a similar form by preparing Triton X-100 mixed micelles containing a 1:1 mixture of both PAPC and lyso-PC substrates. In parallel experiments, we then observed both enzyme activities (Fig. 7). In this dual-substrate assay, AA-TFMK again inhibited cPLA₂, whereas PA-TC showed slightly weaker inhibition. More significantly, under these conditions, inhibition of the PLA₂ activity closely paralleled inhibition of the lysophospholipase activity. It is also interesting that, unlike the single-substrate experiments, under these conditions, the PLA₂ and lysophospholipase activities have similar specific activities. Most important, the surface IC₅₀ (in mole fractions) of AA-TFMK is similar under all of the different experimental conditions (Table II), as would be expected for a true active site-directed inhibitor (19).

**DISCUSSION**

There are a number of characteristic features of the PLA₂ activity of cPLA₂. Among them is an ability to use glycerol as an acceptor of the cleaved fatty acid to generate monoglyceride (11). The presence of glycerol also appears to yield an increase in the measured PLA₂ activity. DAG has been reported to also cause an increase in the PLA₂ activity (4), and activated ketones have been shown to inhibit that activity (19).

In contrast, there is much less known about the lysophospholipase activity of cPLA₂, and what little is known does not appear to be consistent with the mutagenesis data suggesting a common active site for the two activities. For instance, although the PLA₂ activity is known to prefer arachidonic acid-over palmitic acid-containing substrates (23), the rate of lysophospholipase activity has been shown to proceed at similar or greater rates on a palmitic acid-containing substrate than the rate of PLA₂ activity on arachidonic acid-containing substrates (10). The regiospecificity of the lysophospholipase reaction and

---

3 L. J. Reynolds and E. A. Dennis, unpublished data.
the effect of PLA2 activators and inhibitors on the lysophospholipase activity are addressed below.

Regiospecificity—cPLA2 catalyzes the hydrolysis of fatty acid from the sn-2 position of diacylphospholipids to yield a 1-acylglycero-3-phospholipid. In facilitating this reaction, cPLA2 is highly specific for the sn-2 position of diacylphospholipid substrates. It has been reported that no fatty acid is released from the sn-1 position of diacylphospholipids (11). Only when the sn-2 ester was replaced with an unhydrolyzable ether linkage was cPLA2-catalyzed hydrolysis of the remaining sn-1 ester bond detected. However, it was not known from which position cPLA2 catalyzes the hydrolysis of fatty acids from lysophospholipids.

Lysophospholipids can exist as two positional isomers in which the fatty acid is acylated to either the sn-1 or sn-2 position (Fig. 1). In addition, the fatty acyl chain is readily able to migrate between the two hydroxyl groups of the glycerol backbone. At equilibrium, palmitoylglycero-3-phosphocholine exists as a 9:1 mixture of the two isomers, with the palmitic acid predominately at the sn-1 position (20).

In vitro studies of the lysophospholipase activity of cPLA2 typically utilize commercially available 9:1 equilibrium mixtures of palmitoylglycero-3-phosphocholine, and the reaction is generally only followed to a few percent conversion by measuring the formation of palmitic acid (7, 10, 13). Hence, these experiments have not addressed the regiospecificity of the lysophospholipase activity of cPLA2. Indeed, the lysophospholipase activity of cPLA2 could have been due exclusively to the processing of the minor 2-palmitoylglycero-3-phosphocholine isomer by cPLA2, as might be expected for an analogue of its normal diacyl substrate.

We have found that cPLA2 catalyzes the hydrolysis of 1-palmitoylglycero-3-phosphocholine seven times faster than its sn-2 isomer. Thus, the specificity of cPLA2 for the sn-2 position of a diacylphospholipid substrate switches to a preference for the sn-1 position when the substrate is a lysophospholipid. The lysophospholipase sn-2 activity that is observed may in fact be a consequence of 2-PGPC acting as a poor analogue of a diacylphospholipid substrate for the phospholipase A2 activity of cPLA2.

Acyl migration can occur between the two positions of a lysophospholipid and could complicate or obscure our results. However, at pH 7 in the absence of the enzyme, the half-life for migration of palmitic acid between the two hydroxyl groups of GPC is 20 h (20). This is too slow to account for the observed rate of disappearance of 1-PGPC in our experiments; nor is cPLA2
likely to be catalyzing the isomerization of 1-1P-GPC to 2-1P-GPC because the observed consumption of 1-1P-GPC would then have to proceed through a 2-1P-GPC intermediate, and then the observed rate of 1-1P-GPC disappearance could never exceed the rate of 2-1P-GPC disappearance. (At best, the observed rates of disappearance of 1-1P-GPC and 2-1P-GPC would be equivalent in the situation that the hydrolysis reaction is rate-determining.) We find the cPLA<sub>2</sub>-catalyzed rate of 1-1P-GPC disappearance to be 7-fold greater than the rate of 2-1P-GPC disappearance. The above scenario would assume that the isomerization and hydrolysis reactions occur sequentially on the enzyme. In the case that the isomerization and hydrolysis are independent events on the enzyme and intermediate 1P-GPC is released, then the faster rate of 1-1P-GPC disappearance we observed should result in an initial build-up in the amount of 2-1P-GPC and an apparent slower initial rate of 2-1P-GPC disappearance. (If the isomerization step is slower, then the rate of 1-1P-GPC disappearance would remain constant, but the rate of 2-1P-GPC disappearance would initially appear fast as the existing 2-1P-GPC is depleted and would eventually slow to that of 1-1P-GPC disappearance because it is rate-determining.) In fact, the experimentally measured rates of disappearance of both isomers proceed at constant, but different rates. Thus, acyl migration does not appear to be occurring under our experimental conditions and cannot account for the results we observed.

The simplest explanation for the difference in positional specificity between the PL<sub>A</sub> and lysophospholipase activities of cPLA<sub>2</sub> is that there are separate catalytic sites responsible for the two activities. However, there is considerable evidence that both activities share a common catalytic serine residue. The mechanism of cPLA<sub>2</sub> action has been studied by a number of groups, and there are now several pieces of evidence that implicate the involvement of an acyl-enzyme intermediate. Transfer of the fatty acid from both diacyl- and lysophospholipid substrates to acceptors other than water initially suggested the existence of such an acyl-enzyme intermediate (10, 11). Further corroboration came when NMR studies indicated the formation of a hemiketal between arachidonyl trifluoromethyl ketone (a potent inhibitor of both activities) and an enzyme hydroxyl group (24). Finally, a serine residue (Ser-228) was identified to be essential for the PL<sub>A</sub> activity and was also shown to be the site of enzyme acylation (15, 25). This serine residue is required not only for the PL<sub>A</sub> activity, but also for the lysophospholipase activity. The involvement of serine 228 in both activities of cPLA<sub>2</sub> argues against the likelihood of separate catalytic sites for its two activities.

An alternative explanation that accounts for the observed differences in positional specificity of the two activities of cPLA<sub>2</sub> and the common serine required for both activities is that there are different binding pockets on the enzyme for the two substrates that orient positionally different acyl chains toward the shared catalytic serine. This scenario might explain the anomalous report of an antibody to cPLA<sub>2</sub> that inhibits the PL<sub>A</sub> activity and not the lysophospholipase activity (14). The antibody would have to be only blocking a region of the enzyme responsible for binding of diacylphospholipids and leaving free a lysophospholipid-binding site and the catalytic serine.

**Effect of Glycerol, DAG, and Inhibitors**—Based on our earlier studies (26) with a membrane-bound PL<sub>A</sub>, in which glycerol was shown to increase its activity, glycerol was also added to cPLA<sub>2</sub> assays at up to 70% by volume to improve the activity that was measured (2). More recently, it has been shown that glycerol is also able to act as a nucleophile and to accept the fatty acid from a diacylphospholipid substrate (11). In typical radiochemical assays during the isolation of the radiolabeled fatty acid product, the monoglyceride product from the acylation of glycerol also elutes with the free fatty acid formed through the normal hydrolysis pathway, and the two species are counted together. This results in an apparent hydrolysis activity that is greater than what would be measured if the monoglyceride and fatty acid products are separated. Thus, the apparent activity is actually a combination of the hydrolysis and the reaction with water itself. In contrast, with DAG, we have found no evidence for a second product and no activation in a mixed micelle assay.

We have previously shown that activated ketones can inhibit the PL<sub>A</sub> activity of cPLA<sub>2</sub> (19). When we examined these same inhibitors in an assay of the lysophospholipase activity of cPLA<sub>2</sub>, we found that they were behaving quite differently. AA-TFMK inhibits the lysophospholipase activity, but PA-TC and anandamide do not. This suggests that these inhibitors are differentiating between the two cPLA<sub>2</sub> activities. Furthermore, testing these same inhibitors against the PL<sub>A</sub> activity in the DAG assay yielded the same unexpected results. The inhibitors only showed similar effects against both the PL<sub>A</sub> and lysophospholipase activities when examined under identical conditions in which both PAPC and lys-PC substrates were diluted into Triton X-100 mixed micelles. More important, under these conditions, the PL<sub>A</sub> and lysophospholipase activities decreased in parallel in response to the inhibitors. Thus, some facet of the substrate presentation in the different assay conditions appears to affect the ability of the different compounds to inhibit cPLA<sub>2</sub>, rather than inherent differences between the two activities. More important, AA-TFMK inhibits both the PL<sub>A</sub> and lysophospholipase activities with the same surface IC<sub>50</sub> (in mole fractions) in all of the assays examined.

REFERENCES

1. Dennis, E. A. (1994) J. Biol. Chem. 269, 13057–13060
2. Clark, J. D., Milona, N., and Knopf, J. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7708–7712
3. Gronich, J. H., Boventre, J. V., and Nemenoff, R. A. (1990) Biochem. J. 271, 37–43
4. Kramer, R. M., Roberts, E. F., Manetta, J., and Putnam, J. E. (1991) J. Biol. Chem. 266, 5368–5372
5. Takayama, K., Kudo, I., Kim, D. K., Nagata, K., Nozawa, T., and Inoue, K. (1991) FEBS Lett. 282, 326–330
6. Vlijander, J., and Sanders, R. (1994) J. Biol. Chem. 270, 873–880
7. Leslie, C. C. (1991) J. Biol. Chem. 266, 11366–11371
8. Smith, W. L. (1992) Am. J. Physiol. 263, F181–F191
9. Kramer, J. D., Schievelbein, A. R., Naleski, E. A., and Lin, L.-L. (1995) J. Lipid Mediat. Cell Signal. 12, 83–117
10. Reynolds, L. J., Hughes, L. L., Louis, A. I., Kramer, R. M., and Dennis, E. A. (1993) Biochem. Biophys. Acta 1167, 272–280
11. Hanel, A. M., and Gelb, M. H. (1990) Biochemistry 34, 7807–7818
12. Huang, Z., Laliberte, F., Tremblay, N. M., Weech, P. K., and Street, I. P. (1994) Anal. Biochem. 222, 110–115
13. de Carvalho, M. G. S., Garritano, J., and Leslie, C. C. (1995) J. Biol. Chem. 270, 20439–20446
14. Fujinori, Y., Kudo, I., Fujita, K., and Inoue, K. (1995) Eur. J. Biochem. 218, 629–635
15. Sharp, J. D., Pickard, R. T., Chieu, X. G., Manetta, J. V., Kovacevic, S., Miller, J. R., Varshavsky, A. D., Roberts, E. F., Strier, B. A., Brem, D. N., and Kramer, R. M. (1994) J. Biol. Chem. 269, 23250–23254
16. Stafford, R. E. D., and Dennis, E. A. (1988) Colloids Surf. 30, 47–64
17. Pluckthun, A., and Dennis, E. A. (1981) J. Biol. Chem. 256, 678–683
18. Sleight, I. P., Lin, H. K., Laliberte, F., Ghomashchi, F., Wang, Z., Perrier, H., Tremblay, N. M., Huang, Z., Weech, P. K., and Gelb, M. H. (1993) Biochemistry 32, 5935–5940
19. Conde-Frieboes, K., Reynolds, L. J., Lio, Y., Hale, M., Wasserman, H. H., and Dennis, E. A. (1994) J. Am. Chem. Soc. 116, 5519–5525
20. Pluckthun, A., and Dennis, E. A. (1982) Biochemistry 21, 1743–1750
21. Zhang, Y., and Dennis, E. A. (1998) J. Biol. Chem. 263, 9965–9972
22. Brown, H. C., and Fletcher, R. S. (1949) J. Am. Chem. Soc. 71, 1845–1854
23. Wang, Z., Plu¨ckthun, A., and Dennis, E. A. (1996) J. Biol. Chem. 271, 18342–18348
24. Trimble, L. A., Street, I. P., Perrier, H., Tremblay, N. M., Weech, P. K., and Bernstein, M. A. (1993) Biochemistry 32, 2560–2565
25. Huang, Z., Payette, P., Abdulkall, K., Cromlish, W. A., and Kennedy, B. (1996) Biochemistry 35, 3712–3721
26. Ulevitch, R. J., Watanabe, Y., Sano, M., Lieter, M. D., Deems, R. A., and Dennis, E. A. (1988) J. Biol. Chem. 263, 3079–3085