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INACTIVATION OF BEE VENOM PHOSPHOLIPASE A_2 BY A SESQUITERPENE FURANOIC ACID MARINE NATURAL PRODUCT*

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Abstract—A sesquiterpene furanoic acid (SFA) marine natural product isolated from soft corals of the genus *Sinularia* (Bowden *et al.*, *Aust J Chem* **36**: 371–376, 1983) was found to inactivate bee venom phospholipase A_2 (bvPLA$_2$, EC 3.1.1.4) *in vitro*. In this study, we characterized the kinetics of inactivation of bvPLA$_2$ by this compound. The apparent IC_{50} value was 0.5 μM, and the inactivation of bvPLA$_2$ was time dependent. The drug–enzyme binding appeared to be of a non-competitive, high-affinity nature that was irreversible by aqueous dialysis. The inactivation was prevented by the simultaneous addition of excess lysophosphatidylcholine (lysoPC) during the initial binding step, suggesting that modification of the enzyme by SFA occurs at or near the substrate binding site. Activation of bvPLA$_2$ was observed with lysoPC addition at concentrations equimolar to bvPLA$_2$ and higher. Saturation of activation occurred at concentrations greater than 10 μM lysoPC, and preincubation of bvPLA$_2$ with 100 μM lysoPC did not inhibit the enzyme. Analysis of the post-incubation mixture of SFA-inhibited enzyme in the presence of lysoPC revealed the presence of unaltered enzyme exhibiting typical Michaelis–Menten kinetics. The significance of these observations is discussed in light of the recent discussion by Ortiz on the manoalide binding site on bvPLA$_2$ (Ortiz *et al.*, *J Med Chem* **36**: 1866–1879, 1993).

Key words: phospholipase A_2; inflammation; anti-inflammatory; allosteric inhibitors; arthritis

In our laboratory, we have focused on the study of marine natural products as pharmacological probes to explore the various mechanisms by which bvPLA$_2$‡ (EC 3.1.1.4) can be inactivated. These natural products are important probes that can be used to explore the various pathways of eicosanoid biosynthesis at the cellular and molecular level [1–6]. Recent evidence suggests that certain phylogenetically conserved enzymatic pathways, such as cyclooxygenase, lipoxygenase, and cytochrome P450, have been specially adapted to produce novel metabolites from a common substrate [7]. We believe this molecular diversity found in the ocean environment is the consequence of the unique adaptation of the organism to very limited substrates, and thus marine organisms represent a new resource for the discovery of novel anti-inflammatory agents.

In recent years, multiple related and unrelated forms of PLA$_2$ have been discovered from a number of sources, including human, bovine, and rodent tissues, and snake and insect venoms. In addition, considerable effort has been devoted to understanding the fundamental mechanism of phospholipid hydrolysis using the snake and bee venom sources of purified PLA$_2$ as biochemical models [2, 4, 6, 8, 9]. The manoalide binding site on bvPLA$_2$ has been hypothesized recently through molecular modeling to consist of a unique C-terminal extension of residues apparently not present in other forms of PLA$_2$s isolated and characterized thus far [10, 11]. Thus, investigations utilizing bvPLA$_2$ provide an excellent opportunity for the discovery of novel PLA$_2$ inhibitors and an opportunity to begin to understand the fundamental biochemical mechanisms underlying hydrolysis of aggregated phospholipid substrates. These studies will provide new insight into the molecular processes involved in inflammation.

Most of the marine natural products we have studied thus far are hydrophobic isoprenoids that exhibit varying degrees of *in vivo* anti-inflammatory activity in murine models. Some of the putative mechanisms of inactivation of bvPLA$_2$ by marine natural products have been reviewed recently [3]. Thus far, marine natural products possessing a free or masked aldehyde appear capable of selectively reacting with one or more lysine residues at or near the substrate binding site of bvPLA$_2$. The mechanism of inactivation is complex and consists of multiple steps, including a reversible Schiff base reaction

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‡ Abbreviations: PLA$_2$, phospholipase A_2; bvPLA$_2$, bee venom phospholipase A_2; DPPC, dipalmitoylphosphatidylcholine; lysoPC, lysophosphatidylcholine; and SFA, sesquiterpene furanoic acid.

followed by, in some cases, an irreversible pyrole reaction, as reported with manoalide and scalaradial, respectively [2, 12].

Recently, we observed that a sesquiterpene furanoic acid ((1′E,5′Z)-2-(2′,6′-dimethylocta-1,5,7-trienyl)-furan-4-carboxylic acid, SFA), isolated from soft corals of the genus *Sinularia* [13, 14], exhibited significant *in vitro* activity against bvPLA$_2$. Due to the fact that the furan-4-carboxylic acid function of this structure is stable and does not possess a free aldehyde, like manoalide and its analogs [3], we investigated whether this molecule may act by a molecular mechanism not yet described for the known inhibitors of bvPLA$_2$.

The purpose of this report is to identify the biochemical factors controlling the mechanism by which SFA inhibits bvPLA$_2$ and describe the inactivation kinetics and reversibility of the drug–enzyme complex.

MATERIALS AND METHODS

Materials

L-α-Dipalmitoyl phosphatidylcholine, lysophosphatidylcholine, HEPES, bvPLA$_2$ (1300 U/mg protein), and Triton X-100 were obtained from the Sigma Chemical Co. (St. Louis, MO). L-α-Dipalmitoyl-[2-palmitoyl-9,10-^{3}H(N)]phosphatidylcholine (sp. act. 50 Ci/mmol) and [1-^{14}C]palmitic acid (sp. act. 8.4 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Silica gel 60 (HR extra pure) was purchased from EM Science (Cherry Hill, NJ). Biosafe scintillation fluid was obtained from Research Products (Mount Prospect, IL).

PLA$_2$ radioassay

Phosphatidylcholine substrate was prepared at 1.36 mM as mixed micelles. Briefly, unlabeled L-α-dipalmitoyl phosphatidylcholine was homogenized in 10 mM HEPES buffer, pH 7.4, at 41°, containing 0.15% (w/v) Triton X-100 and various amounts of $CaCl_2$ (0 to 5 mM) depending on the specific assay. ^{3}H-Labeled L-α-dipalmitoyl phosphatidylcholine was added to a separate test tube and organic solvent was removed. Unlabeled substrate solution was added, and the solution was sonicated until clear in an ice bath for 30–45 min (final activity of 0.25 μCi/0.5 mL substrate). Bee venom PLA$_2$ (100 U/mL) was prepared in 10 mM HEPES–$CaCl_2$ buffer, pH 7.4, at 41° (approx. 12 μM as determined by the Bradford protein assay [15] using bovine gamma globulin as the protein standard). SFA was dissolved in methanol. The final methanol concentration in drug and control tubes was 1.6% (v/v) before dilution into substrate. SFA/enzyme mixtures were incubated in triplicate at 41° for 1 hr unless otherwise noted. Enzyme activity was assayed in triplicate under conditions previously described [16]. Briefly, 5 μL of SFA/enzyme mixture was added to 0.5 mL of substrate at 41° (final enzyme concentration of 0.12 μM with substrate). After 15 sec at 41°, the reaction was terminated with 2 mL isopropyl alcohol/heptane/0.5 M sulfuric acid (40:10:1, by vol.). Free palmitic acid was extracted as follows. Heptane (2 mL) and water (1 mL) were added, and tubes were vortexed and centrifuged at 1200 *g* for 10 min. Then 2 mL of the heptane layer was removed and added to tubes containing 150 mg silica. After vortexing and centrifugation at 1200 *g* for 15 min, 1 mL was removed and added to 3 mL of Biosafe scintillation fluid and counted in an LKB scintillation counter. Extraction efficiency was determined by co-extraction of ^{14}C-labeled palmitic acid in tubes containing no enzyme. In concentration–response studies, 12 μM bee venom PLA$_2$ was preincubated with varying concentrations of SFA for 1 hr, and enzyme activity was assayed by 100-fold dilution into 1.36 mM DPPC. All solutions were in 10 mM HEPES, 1 mM $CaCl_2$ buffer, pH 7.4, at 41°.

Substrate and enzyme kinetic studies

Bee venom PLA$_2$ (12 μM) was preincubated with SFA (100 μM) for 1 hr, and enzyme activity was assayed by 100-fold dilution into varying substrate concentrations. Final concentrations were 1 μM SFA and 0.12 μM bvPLA$_2$. For the studies on the effects of enzyme concentration on SFA inactivation, 100 μM SFA was incubated with varying molar concentrations of bvPLA$_2$ for 1 hr, and enzyme activity was assayed by 100-fold dilution into 1.36 mM DPPC. All solutions were in 10 mM HEPES, 5 mM $CaCl_2$ buffer, pH 7.4, at 41°.

Progressive inactivation and dialysis studies

The rate of inactivation of bvPLA$_2$ by the SFA was determined by preincubating the compound (100 μM) with 12 μM enzyme for various time intervals (15 sec to 1 hr). Enzyme activity was assayed by 100-fold dilution into 1.36 mM DPPC. Dialysis was performed on the remaining 60-min preincubation mixtures by pooling the triplicate tubes and placing 1 mL of SFA/enzyme mixture into SpectraPor mol. wt 10,000 cut-off dialysis tubing. The mixture was dialyzed against 1 L of buffer at 4° for 24 hr with two buffer changes before post-dialysis enzyme activity analysis. All solutions were in 10 mM HEPES, 5 mM $CaCl_2$ buffer, pH 7.4, at 41°.

Reversal of inactivation by lysoPC

Lysophosphatidylcholine (lysoPC) was added as a buffer dispersion in molar excess (1×, 10×, and 100×) over SFA concentration during the 8-min preincubation of 100 μM SFA and 12 μM bvPLA$_2$ in 10 mM HEPES buffer, pH 7.4, containing no added calcium, at 41°. Enzyme activity was assayed by 100-fold dilution into 1.36 mM DPPC in 10 mM HEPES, 5 mM $CaCl_2$ buffer as described earlier. Final concentrations were 1 μM SFA, 0.12 μM enzyme, and 1, 10, and 100 μM lysoPC.

RESULTS

Concentration-dependent inactivation of bvPLA$_2$ by SFA

SFA significantly inactivated bvPLA$_2$ (Fig. 1). As can be seen, a concentration-dependent inactivation occurred over 3 log U with an apparent IC_{50} of 0.5 μM. The slope of the concentration–response curve was qualitatively different from that obtained

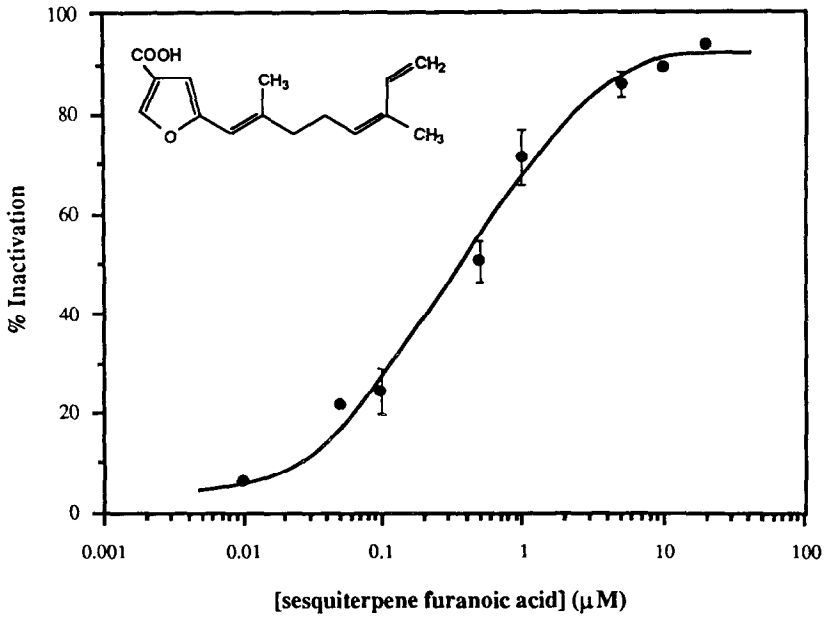

Fig. 1. Inhibition of bvPLA$_2$ by SFA. SFA was preincubated with bvPLA$_2$ for 1 hr at 41° in 1 mM $CaCl_2$, 10 mM HEPES buffer, pH 7.4. Enzyme activity was assayed as detailed in Materials and Methods. SFA concentrations shown are the final concentrations. Data with error bars represent means ± SEM (N = 3) and data without error bars represent means (N = 2) of percent inactivation as compared with controls. Control enzyme activity = 0.23 to 0.38 μmol/min.

with manoalide or scalaradial [2, 16], in that the linear portion occurred over 2 log U. This suggests that SFA may interact with different affinity binding sites on bvPLA$_2$. The IC_{50} value of 0.5 μM for SFA was also approximately 10-fold less potent than that obtained for manoalide and scalaradial. A double-reciprocal plot of the concentration-dependent inactivation data was non-linear (data not shown), supporting the observation that the inactivation was complex and did not follow pseudo-first-order kinetics.

Irreversible properties

When 100 μM SFA was preincubated with 12 μM enzyme for 1 hr at 41°, the enzyme was inactivated by 72% compared with control samples. Following aqueous dialysis for 24 hr against a 1000-fold dilution with HEPES–$CaCl_2$ buffer at 4°, no reversal of inactivation was detected when compared against control samples dialyzed simultaneously (data not shown). Post-dialysis control samples retained 48% of activity when compared to pre-dialysis controls, and post-dialysis SFA-treated samples retained 56% of activity when compared to pre-dialysis SFA-treated samples. The loss of activity in control and SFA-treated samples was paralleled by an approximately equivalent loss in protein content after dialysis as determined by Bradford analysis [15]. This suggests that under the conditions of these experiments, SFA inhibition was apparently irreversible or perhaps was the result of high affinity hydrophobic binding not easily dissociated by aqueous buffer.

Further evidence that SFA inactivation of bvPLA$_2$ was apparently irreversible was based on the analysis of experiments with varying concentrations of bvPLA$_2$ in the presence and absence of a fixed concentration of SFA (Fig. 2). As can be seen, there was an approximate parallel shift in the lines, indicating a constant reduction in the amount of available or free enzyme, which is typical of irreversible enzyme inhibitors. The slope of the lines and thus the specific activity of the uninhibited enzyme were statistically identical in the presence and absence of SFA.

Kinetics of inactivation

Progressive inactivation of PLA$_2$. When 100 μM SFA was preincubated with 12 μM PLA$_2$ at 41°, the loss of bvPLA$_2$ activity was progressive with time (Fig. 3). As can be seen, the rate of inactivation was relatively slow, with 75% of the maximal obtainable inactivation for this concentration of SFA occurring within the first 8 min of preincubation. The loss of bvPLA$_2$ activity was non-linear with respect to time on a semilogarithmic plot, indicating that the reaction does not follow pseudo-first-order kinetics under these conditions. Also, the time-dependent inactivation was typical of irreversible inhibitors or inhibitors in which the binding is a slow process that does not exhibit typical Michaelis–Menten kinetics.

Effects on substrate binding. Figure 4 illustrates a series of experiments in which the inhibition by preincubation with SFA was assessed at various substrate concentrations. In the control curve, typical pseudo-first-order kinetics were observed. The

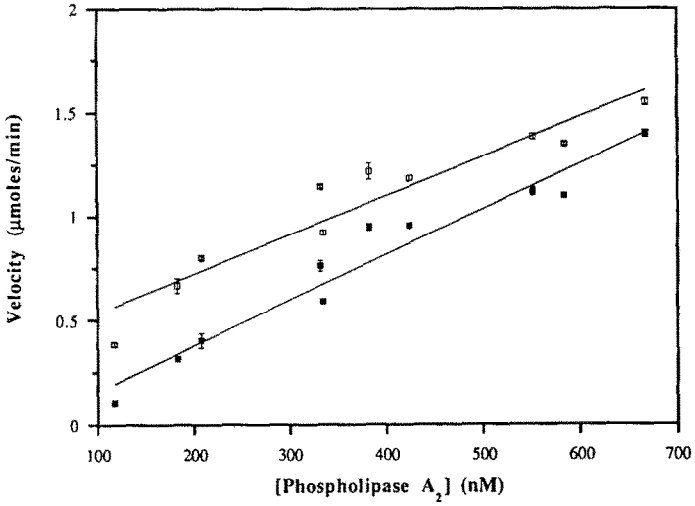

Fig. 2. Inactivation by SFA as a function of bvPLA$_2$ concentration. SFA (100 μM) was preincubated with increasing molar concentrations of enzyme for 1 hr at 41° in 5 mM $CaCl_2$, 10 mM HEPES buffer, pH 7.4, before dilution of an aliquot into the substrate. Enzyme concentrations shown are final concentrations in the assay. Key: (□) control and (■) enzyme treated with 1 μM SFA. Data are means ± SEM (N = 3). The difference between the slopes was not significant, $P \geq 0.05$ (Student's t-test).

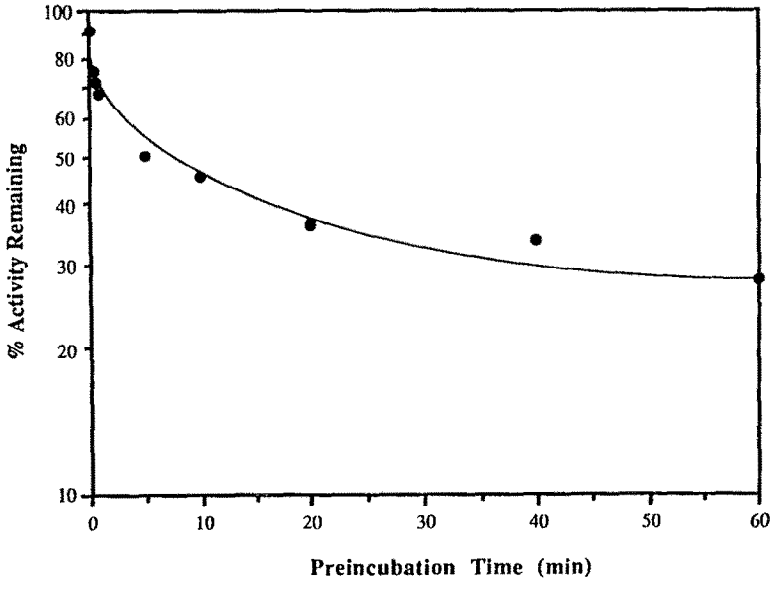

Fig. 3. Progressive inactivation of bvPLA$_2$ by SFA. SFA (100 μM) was preincubated with 12 μM enzyme for various times at 41° in 5 mM $CaCl_2$, 10 mM HEPES buffer, pH 7.4. Enzyme activity was assayed as detailed in Materials and Methods. Final concentrations: 1 μM SFA and 0.12 μM enzyme.

specific activity of the enzyme increased with increasing substrate concentrations and began to exhibit substrate saturation at 4 mM DPPC. The apparent K_m for the substrate was 1.07 mM and the apparent V_{max} reached 0.37 μmol/min/μg protein by Lineweaver–Burk analysis (see Fig. 4 inset). Following preincubation with SFA, the substrate binding curve also showed saturation; however, the initial slope of the curve and the plateau indicate marked quantitative changes with respect to the residual enzyme activity. If we have irreversibly inhibited the enzyme, the residual uninhibited enzyme should have kinetics identical to those of the control with respect to the apparent K_m but not with respect to the V_{max}. A double-reciprocal plot of the data is shown in Fig. 4 (inset) and, as can be

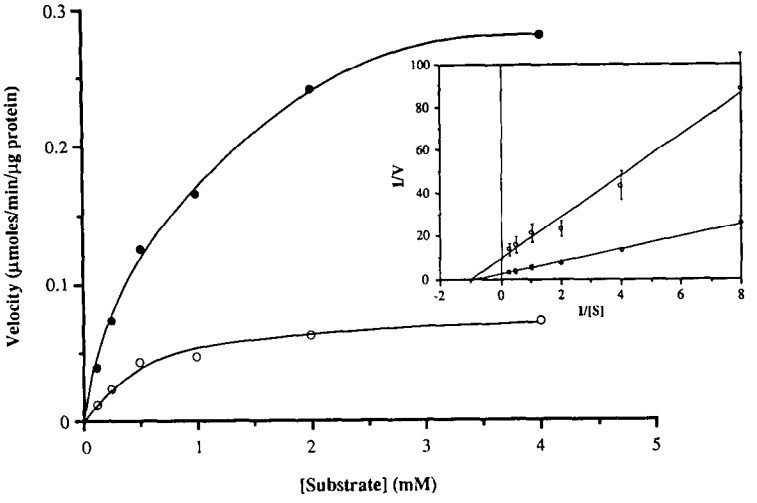

Fig. 4. Effects of increasing substrate concentrations on SFA inactivation of $bvPLA_2$. SFA (100 μM) was preincubated with 12 μM enzyme for 1 hr at 41° in 5 mM $CaCl_2$, 10 mM HEPES buffer, pH 7.4, before dilution of an aliquot into increasing substrate concentrations. Final SFA concentration was 1 μM with 0.12 μM enzyme. Data are means ± SEM (N = 3). Inset: Lineweaver–Burk analysis. Key: (●) control and (○) treated enzyme activity.

seen, the apparent K_m for the substrate remained about the same (1.01 mM) following preincubation with SFA. V_{max}, however, was decreased to 0.105 μmol/min/μg protein. This is consistent with a model of apparent noncompetitive or mixed inhibition. Because SFA was an apparent irreversible inhibitor of $bvPLA_2$, classical Lineweaver–Burk analysis was not usually indicated. However, the fact that the SFA was preincubated with the enzyme and then diluted 100-fold into the substrate allows for Lineweaver–Burk analysis of the residual uninhibited enzyme. Since the kinetics of inactivation and formation of the irreversible complex was complete, the analysis of the residual activity was valid and is evidence that SFA does not act by altering substrate affinity.

Effects of lysoPC on SFA inactivation

To investigate whether SFA was binding at or near the substrate binding site, we attempted to protect the enzyme from inhibition or prevent the inhibition by preincubating the enzyme with SFA in the presence of excess buffer-dispersed lysoPC, a hydrolysis product. LysoPC was added in molar excess (1×, 10×, and 100×) relative to SFA during the initial enzyme inactivation period for 8 min. These preincubation mixtures were calcium free. As previously shown, 75% of the maximal obtainable inactivation by SFA at this concentration occurred during the first 8 min of preincubation. The assay of activity was performed following 100-fold dilution into substrate. As can be seen in Fig. 5, addition of lysoPC in concentrations from 1 to 100 μM increased the specific enzymatic control activity up to 23%. This activation exhibited saturation above 10 μM lysoPC. The activation observed, although not statistically significant (Student's t-test = 2.4, 2 df), was consistent and reproducible, and further experiments are underway to validate this activation phenomenon. LysoPC did not inhibit control hydrolysis rates, indicating that there was no competitive antagonism with respect to DPPC at these concentrations. This putative activation phenomenon may represent a unique process, perhaps similar to a type of positive cooperativity, where binding of the hydrolysis product to a site at or near the substrate binding site activates the enzyme.

In the presence of SFA, excess lysoPC prevented the inactivation of the enzyme or perhaps protected it from inactivation in the first place. The reversal of the percent inhibition reached a maximum of 80% but did not increase significantly beyond 10 μM lysoPC. The maximum protection obtained was less than the maximum velocity of the activated control enzyme, but was about equal to the control enzymatic activity level obtained in the absence of lysoPC.

DISCUSSION

Mode of action of SFA

In the experiments reported here, SFA was shown to be a reasonably potent inhibitor of $bvPLA_2$, with an apparent IC_{50} of 0.5 μM. The flattened slope of the log concentration–response curve obtained for SFA suggests that this drug may interact with multiple affinity binding sites on $bvPLA_2$. The inactivation of the enzyme was time dependent and, based on dialysis experiments in aqueous medium, the inhibition was apparently irreversible. Studies on the drug–enzyme complex showed that although the SFA inactivation was not reversible by dialysis, we were able to prevent inactivation by SFA during the first 8 min of preincubation by the addition

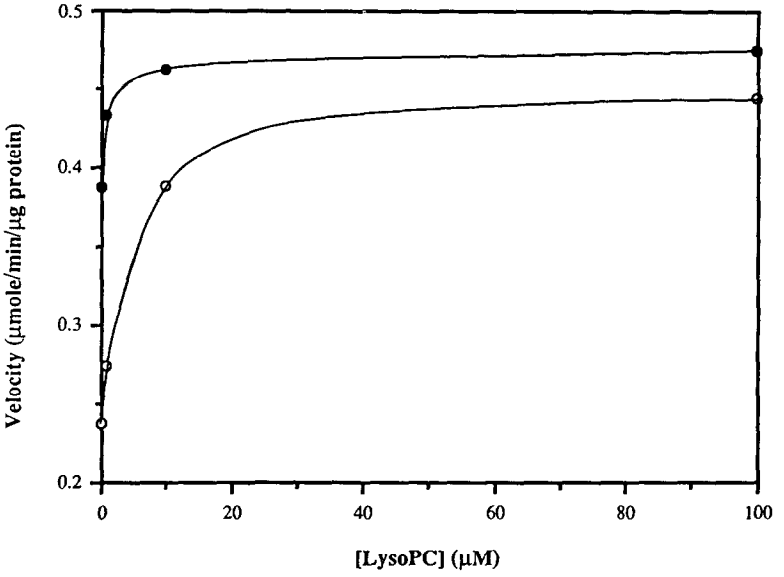

Fig. 5. Effects of preincubation of lysoPC in the absence of buffer calcium on control and SFA-treated enzyme activity. LysoPC was added in molar excess (1×, 10×, and 100×) over SFA concentration during the 8-min preincubation of 100 μM SFA and 12 μM enzyme in 10 mM HEPES buffer. Enzyme activity was assayed as detailed in Materials and Methods in 5 mM $CaCl_2$, 10 mM HEPES buffer. Final concentrations: 1 μM SFA and 0.12 μM enzyme. Key: (●) control and (○) SFA-treated enzyme activity.

of buffer-dispersed lysoPC to the preincubation medium. Although the exact mechanism is not understood at this time, we have made two observations based on our results: (1) kinetic analysis indicates that SFA may inhibit $bvPLA_2$ by an irreversible and non-competitive mechanism, and (2) lysoPC prevented SFA inhibition and may also directly activate $bvPLA_2$. As far as we are able to determine at this time, a PLA_2 inhibitor having the same mode of action as SFA has not been reported previously. In addition, our study raises the possibility that there is a site(s) on the $bvPLA_2$ molecule sensitive to lysoPC activation.

Site of action of SFA

Definitive studies of the site of action of SFA are incomplete at this time. We do not have direct evidence that SFA binds to the manoalide binding site; however, the rate of inactivation was relatively slow, indicating that the drug–enzyme complex forms slowly as expected when the binding involves hydrophobic interactions. Also, the drug binding may involve a putative lysoPC binding site. Since the SFA–$bvPLA_2$ complex could not be dissociated with high concentrations of aggregated DPPC but was prevented from forming with a high concentration of an aqueous dispersion of lysoPC, the possibility arises that a distinct lysoPC binding site exists, separate from the catalytic site and perhaps separate from but proximal to the substrate binding site. As pointed out by Blow [11], there are many unanswered questions involved in determining what conformational changes, if any, are required for the PLA_2 enzymes to hydrolyze aggregated phospholipid substrates. Similarly, there is little information as yet on what happens to the products of hydrolysis. For example, is there a translocation of the product, lysoPC, from a point proximal to the catalytic site to a point proximal to the activation site? This would explain our lysoPC activation data. In the experiments reported here, both lysoPC and SFA were allowed to react with $bvPLA_2$ in a non-aggregated, soluble form in the absence of calcium. Activation could be a direct process that does not require reaction at the phospholipid bilayer interface.

Bee venom PLA_2 inhibitors

In the recent literature, there have been several investigations of various classes of PLA_2 inhibitors. Thus, PLA_2 inhibitors have been reported on the basis of chemical class (e.g. Schiff base inhibitors) [12]; on the basis of functional site on the enzyme (e.g. catalytic site inhibitors such as *p*-bromophenacyl bromide) [17, 18]; suicide inhibitors [19]; and transition state inhibitors [20]. Inhibitors that bind to a site but do not inhibit the catalytic site include manoalide and manoalogue, and other drugs, which may act to displace part of the interfacial substrate recognition site [6]. The drug binding site for these inhibitors, which may be the same site for SFA and lysoPC, may be ultimately involved in the process of enzymatic activation rather than substrate catalysis, since it has been shown recently that phosphocholine analogs can both stimulate and inhibit hydrolysis [21]. It makes sense to us that lysoPC would be a natural activator of PLA_2. Furthermore, lysoPC should contain the minimum functional groups and molecular architecture to optimally fit the substrate binding site but not the catalytic site on the enzyme.

Inhibition by aggregated substrate analogs

When PC analogs are presented as substrate aggregates, they have been shown to activate cobra venom PLA_2 at low concentrations and inhibit at high concentrations, producing a unique biphasic curve [21]. In contrast, we preincubated $bvPLA_2$ with SFA and an aqueous dispersion of lysoPC in our studies. The activation of the enzyme in our studies was probably not mitigated by either the addition of aggregated DPPC after preincubation or by the presence of excess lysoPC because in these experiments, the hydrolysis reaction was allowed to proceed for only 15 sec and the substrate micelles are already solubilized with Triton X-100 and are optimized and stabilized by sonication before running the reaction.

Comparison of SFA with scalaradial and manoalide

Certain features of SFA inhibition are reminiscent of manoalide and scalaradial. The structural similarities between these two previously discovered drugs have been compared recently by molecular modeling with the X-ray structure of $bvPLA_2$, and it appears that these two drugs share a common binding site and a partially similar mechanism of action [10]. Also, the authors point out that one additional positive charge in the enzyme may be required in order to interact with the carboxyl group formed during the opening of the furan ring in manoalide. SFA has an available free carboxyl group that may also react with this putative positively charged site in the enzyme.

Another similarity does exist with respect to manoalide and scalaradial, in that we have shown previously that the addition of excess aqueous-dispersed lysoPC or DPPC to the preincubation medium will prevent manoalide and scalaradial inactivation, respectively [2, 22]. Thus, the data raise the possibility that manoalide may also bind to a similar site as part of its complex inactivation of $bvPLA_2$.

Conclusions

SFA is a relatively rare marine natural product that inactivates $bvPLA_2$ in a complex manner and possibly binds to more than one site on $bvPLA_2$. Its apparent irreversible inactivation of $bvPLA_2$ is interesting given that its molecular structure does not have any chemical substituents that would be obviously reactive to form an irreversible complex with the enzyme similar to that described for manoalide, scalaradial, and their analogs. However, the binding of SFA to $bvPLA_2$ is a relatively slow process, perhaps involving some hydrophobic interactions, similar to those observed with manoalide and scalaradial. Because of the carboxylic acid substitution on the furan ring, Schiff base inactivation appears unlikely. By way of speculation, it is possible that the free ionized carboxyl group on the furan ring may form an initial ionic interaction with a positive charge proximal to the hydrolysis site in the enzyme, thus pulling the drug into its binding site and providing a mechanism for the inhibition observed. This binding site appears to be at least part of the putative lysoPC activation site on the enzyme.

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REFERENCES

1. Jacobson PB, Marshall LA, Sung A and Jacobs RS, Inactivation of human synovial fluid phospholipase A_2 by the marine natural product manoalide. *Biochem Pharmacol* **39**: 1557–1564, 1990.
2. de Carvalho MS and Jacobs RS, Two-step inactivation of bee venom phospholipase A_2 by scalaradial. *Biochem Pharmacol* **42**: 1621–1626, 1991.
3. Potts BCM, Faulkner DJ and Jacobs RS, Phospholipase A_2 inhibitors from marine organisms. *J Nat Prod* **55**: 1701–1717, 1992.
4. Mayer AMS, Paul VJ, Fenical W, Norris JN, de Carvalho MS and Jacobs RS, Phospholipase A_2 inhibitors from marine algae. *Hydrobiologia* **260/261**: 521–529, 1993.
5. Glaser KB, Mobilio D, Chang JY and Senko N, Phospholipase A_2 enzymes: Regulation and inhibition. *Trends Pharmacol Sci* **14**: 92–98, 1993.
6. Reynolds LJ, Mihelich ED and Dennis EA, Inhibition of venom phospholipases A_2 by manoalide and manoalogue. Stoichiometry of incorporation. *J Biol Chem* **266**: 16512–16517, 1991.
7. Jacobs RS, Bober M, Pinto I, Williams AB, Jacobson PB and de Carvalho MS, Pharmacological studies of novel marine metabolites. In: *Advances in Marine Biotechnology*, (Eds. Attaway DH and Zaborsky OR), Vol. 1, pp. 77–99. Plenum Press, New York, 1992.
8. Glaser KB, de Carvalho MS, Jacobs RS, Kernan MR and Faulkner DJ, Manoalide: Structure–activity studies and definition of the pharmacophore for phospholipase A_2 inactivation. *Mol Pharmacol* **36**: 782–788, 1989.
9. Jacobs RS, Culver P, Langdon R, O'Brien T and White S, Some pharmacological observations on marine natural products. *Tetrahedron* **41**: 981–984, 1985.
10. Ortiz AR, Pisabarro MT and Gago F, Molecular model of the interaction of bee venom phospholipase A_2 with manoalide. *J Med Chem* **36**: 1866–1879, 1993.
11. Blow D, Lipases reach the surface. *Nature* **351**: 444–445, 1991.
12. Potts BCM, Faulkner DJ, de Carvalho MS and Jacobs RS, Chemical mechanism of inactivation of bee venom phosopholipase A_2 by the marine natural products manoalide, luffariellolide and scalaradial. *J Am Chem Soc* **114**: 5093–5100, 1992.
13. Coll JC, Mitchell SJ and Stokie GJ, Studies of Australian soft corals. V. A novel furanosesquiterpene acid from the soft coral *Sinularia gonatodes* (Kolonko). *Tetrahedron Lett* **18**: 1539–1542, 1977.
14. Bowden BF, Coll JC, de Silva ED, de Costa MSL, Djura PJ, Mahendran M and Tapiolas DM, Studies of Australian soft corals. XXXI. Novel furanosesquiterpenes from several soft corals (*Coelenterata, Octocorallia, Alcyonacea*). *Aust J Chem* **36**: 371–376, 1983.
15. Bradford M, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
16. Glaser KB and Jacobs RS, Molecular pharmacology of manoalide inactivation of bee venom phospholipase A_2. *Biochem Pharmacol* **35**: 449–453, 1986.
17. Roberts MF, Deems RA, Mincey TC and Dennis EA, Chemical modification of the histidine residue in phospholipase A_2 (*Naja naja naja*). *J Biol Chem* **252**: 2405–2411, 1977.
18. Volwerk JJ, Pieterson WA and de Haas GH, Histidine

at the active site of phospholipase A_2. *Biochemistry* **13**: 1446–1454, 1974.
19. Washburn WN and Dennis EA, Suicide-inhibitory bifunctionally linked substrates (SIBLINKS) as phospholipase A_2 inhibitors. *J Biol Chem* **266**: 5042–5048, 1991.
20. Scott DL, White SP, Otwinowski Z, Yuan W, Gelb MH and Sigler PB, Interfacial catalysis: The mechanism of phospholipase A_2. *Science* **250**: 1541–1546, 1990.
21. Yu L, Deems RA, Hajdu J and Dennis EA, The interaction of phospholipase A_2 with phospholipid analogues and inhibitors. *J Biol Chem* **265**: 2657–2664, 1990.
22. Glaser KB, Molecular pharmacology of the marine natural product manoalide: Irreversible inactivation of bee venom phospholipase A_2. *Ph.D. Dissertation*, University of California, Santa Barbara, 1987.