MATERIALS AND METHODS

Folding of Synthetic GV-PLA_2

Lyophilized synthetic GV-PLA_2 protein (200 μg) was dissolved in 50 ml of 25 mM Tris-HCl (pH 8.5) containing 2 M guanidine hydrochloride. The diluted protein solution was then dialyzed against 4 L of 25 mM Tris-HCl (pH 8.5) buffer containing 1 M guanidine hydrochloride overnight to gradually reduce the concentration of guanidine hydrochloride. The dialysis buffer was changed 4 times during this period. The folding process was initiated by adding $CaCl_2$ to 10 mM and cysteine to 5 mM final concentration. The folding of synthetic GV-PLA_2 was achieved by stirring at 4 °C for 48 h. The protein sample was dialyzed against 4 L of 25 mM Tris-HCl (pH 8.0) buffer to remove guanidine hydrochloride.

PLA_2 Activity Assay

The PLA_2 assay mixture consisted of 100 μM DPPC containing 100,000 cpm radiolabelled with ^{14}C in the *sn*-2 position, 5 mM $CaCl_2$, and 50 mM Tris-HCl (pH 8.5) in a final volume of 500 μl. The mixture of cold and radiolabelled DPPC was dried under a stream of nitrogen. The dried phospholipid was then suspended in an appropriate volume of the assay buffer. The vesicle substrate was formed by sonicating and vortexing until the solution clarified. The mixed micelle substrate was prepared by adding appropriate volumes of Triton X-100 stock solution so as to achieve a molar ratio of DPPC-Triton (1:5) and warming at 50 °C for 10 min with vortexing. Assays were initiated by the addition of 10-50 ng of GV-PLA_2 and were run at 40 °C for 30 min with agitation. The reaction was quenched by adding 2.5 ml of Dole reagent [1] (Isoproponal/heptane/0.5 M H_2SO_4, 20:5:1 v/v). The amount of released radiolabelled fatty acids was subsequently determined according to a modified [2] Dole

extraction procedure as described previously [3]. Blank reactions were routinely performed and subtracted from the reported activities.

Other Methods

Protein concentrations were determined with the Bradford protein microassay using bovine serum albumin as the standard [4].

RESULTS AND DISCUSSION

Folding of Synthetic GV-PLA_2

The synthetic protein was folded by incubation with Ca^{2+} and cysteine. After removal of quanidine hydrochloride, 140 μg of active synthetic GV-PLA2 was obtained from 200 μg synthetic protein. To prevent aggregation and precipitation during the folding process, the protein concentration was kept at a very low level (4 μg/ml), resulting in successful folding and a relatively high yield (70%) for this step. The active enzyme was a pure protein with molecular weight of about 14 kDa on SDS-PAGE (data not shown).

PLA_2 Activity of Synthetic GV-PLA_2

The activity of the folded synthetic GV-PLA2 toward DPPC-Triton mixed micelles and DPPC sonicated vesicles was examined. In both assay systems, the activity dependence on protein is linear up to at least 5% hydrolysis of the phospholipid as shown in Fig. 1.

Comparison of the GV-PLA_2 obtained from bacterial expression [5] with the synthetic enzyme reveals exactly the same specific activity for the DPPC-Triton mixed micellar substrate. However, when the DPPC sonicated vesicle substrate was used, the specific activity of the synthetic enzyme is slightly lower than the bacterially expressed enzyme as

indicated in Table I. It should be noted that the bacterially expressed enzyme contains four additional extra amino acid residues at the N-terminus and these could alter slightly the interaction with vesicles, but not have any effect on mixed micelles; this is under investigation.

REFERENCES

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Figure Legends

Figure 1. Dependence of activity of folded synthetic GV-PLA2 toward DPPC/Triton X-100 mixed micelles (o) and sonicated vesicles (•). The lines are linear regressions.

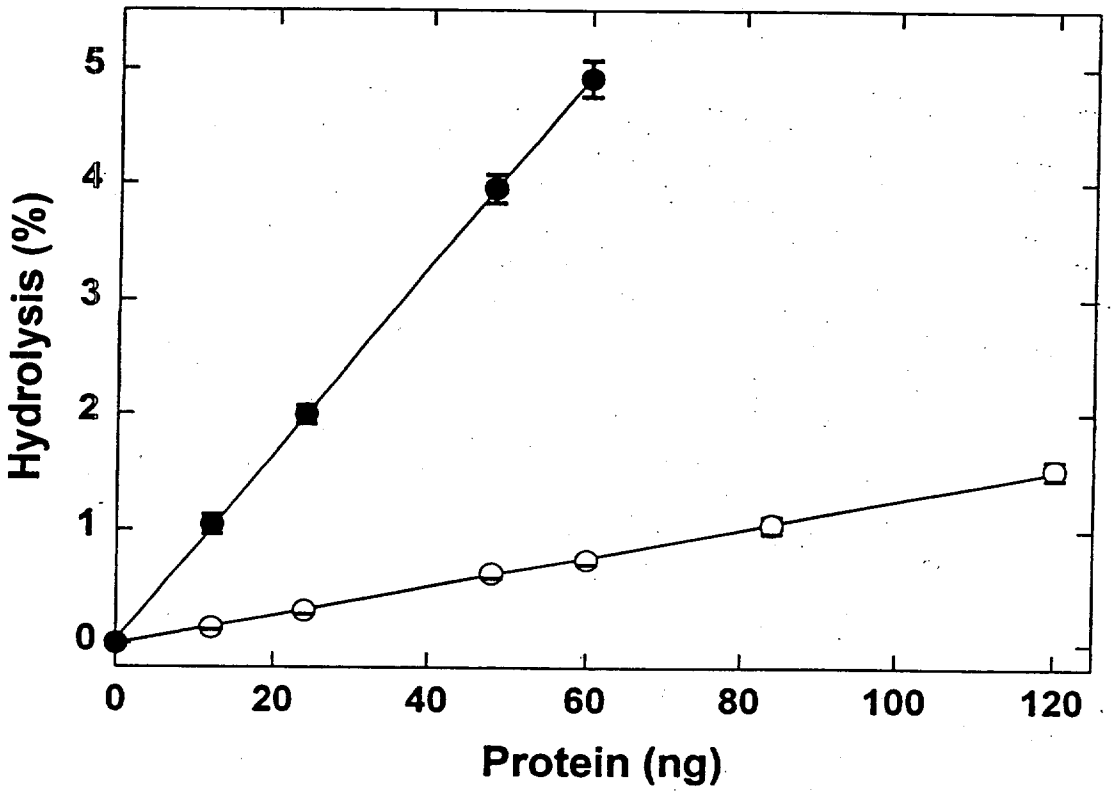
Hydrolysis (%)
0
1
2
3
4
5
0
20
40
60
80
100
120
Protein (ng)

Table I. Comparison of the Specific Activities Chemically Synthesized and Bacterially Expressed GV-PLA_2s[a]

Enzyme	Mixed Micelles (μmol/min/mg)	Sonicated Vesicles (μmol/min/mg)
Synthetic	0.3 ± 0.02	2.5 ± 0.12
Bacterial	0.3 ± 0.01	3.2 ± 0.14

[a]Values are the mean and standard deviation of four measurements in two separate experiments.