The interaction of a polymeric persimmon proanthocyanidin fraction with Chinese cobra PLA2 and BSA

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Article history:
Received 26 November 2012
Received in revised form 28 February 2013
Accepted 6 March 2013
Available online 14 March 2013

Keywords:
Persimmon tannin
PLA2
Structure modification
Covalent modification
Protein affinity
BSA

1. Introduction

Snake venoms are complex mixtures of proteins including phospholipase A2 (PLA2), myotoxins, hemorrhagic metalloproteases and other proteolytic enzymes (Ushanandini et al., 2006). The toxicity of snake venom involves a complex combined action of these venom components. Effective agents for inhibiting venoms include antiserum, chemical antidotes, and anti-snake venom proteins. In addition, some plant natural products including tannins, which are naturally occurring polyphenolic compounds, have been reported to have significant protective effect against snake venom. In vitro studies of venom neutralization and in silico molecular docking indicated that plant polyphenols from the aqueous extracts of Pentace burmanica, Pithcellobium dulce, Areca catechu or Quercus infectoria significantly inhibited Naja kaouthia venom by selectively blocking the nicotinic acetylcholine receptor and by non-selectively precipitating the venom proteins (Pithayanukul et al., 2005). Although some tannins have promising activities against snake venom in vitro, these activities are not always manifested in vivo (Borges et al., 2005). However, the aqueous extract of Schizolobium para-hyba (Caesalpinoideae), which is rich in polyphenols, neutralizes snake venom in vivo when administered by the same route some time after venom injections (Mendes et al., 2008). Pithayanukul et al. (2010) found that tea polyphenols inhibited the hemorrhagic and the dermo-necrotic activities of the venoms in vivo due to complexation and chelation between the venom proteins and the phenolic contents of the extract (Pithayanukul et al., 2010). The mechanism of action of polyphenols against venoms is not fully understood, but may involve interaction between the polyphenol and the venom protein.
The polyphenols classified as tannins characteristically interact strongly with proteins, forming precipitates under favorable stoechiometric conditions (Hagerman, 2012). The interaction of tannins with proteins is essential to their biological activities (Frazier et al., 2010). For example, the interactions of the tea polyphenol, epigallocatechin gallate (EGCG), with proteins such as fatty acid synthase (FAS) (Hayakawa et al., 2001), laminin (Suzuki and Isemura, 2001), the 67-kDa laminin receptor (67LR) (Tachibana et al., 2004) or the glucose-regulated protein 78 (Ermakova et al., 2006) have been proposed to be related to the anticancer effects of EGCG. The dimeric procyanidin B2 was reported to interact with NF-kappa-B and to prevent the binding of NF-kappa-B to the DNA kappa-B sites, preventing expression of inflammatory proteins (Mackenzie et al., 2009). Direct interaction of red wine polyphenols with the pancreatic lipase active site has been reported, and may be associated with the anti-atherosclerotic activity of red wine (Sarra et al., 2005).

The mechanism of action of some polyphenols involves covalent modification of the active protein. For example, EGCG irreversibly inhibits glyceraldehyde-3-phosphate dehydrogenase by covalently modifying the cytoeinst thiol group in the active center (Ishii et al., 2008). EGCG and ECG are readily oxidized to form semiquinones or quinones, which can bind covalently with some nucleophilic functional groups of protein including lysine, histidine, cysteine, and tyrosine (Chitpan et al., 2007), thus irreversibly altering the structure and function of the protein.

In studying the biological activities of persimmon tannin (PT), which is a high molecular weight, highly galloylated condensed tannin with both A and B type linkages and an unusual galloylated condensed tannin with both A and B type linkages and an unusual galloylated condensed tannin, we speculated that covalent complexes might form between PT and PLA2. We examined those interactions by chemically modifying the structure of PLA2 and examining the redox-cycling staining. We speculated that more detailed comparisons of the interactions between BSA or PLA2 with PT40 would aid in better understanding the anti-venom mechanism of persimmon tannin in vivo.

2. Materials and methods

2.1. Materials

Chinese cobra (Naja naja atra) Phospholipase A2 (PLA2, 9001-84-7) was purchased from ZhongXin DongTai Nano Gene Biotechnology Co. Ltd. (Laiyang, China). The PLA2 yielded a single homogeneous band on SDS-PAGE electrophoresis and a single peak on the HPLC (Zorbax 300SB-C18 column, with a linear gradient acetonitrile (0–40%) in 0.1% trifluoroacetic acid), indicating a purity of more than 95% and a molecular weight of 14,400 Da. Bovine serum albumin (BSA, Fraction V, fatty acid free) was from Sigma (St. Louis, MO). Persimmon tannin fraction PT40 was prepared and characterized as we previously reported (Li et al., 2010; Xu et al., 2012; Yang et al., 2012). The content of total polyphenols in HMWPT was 98.7% on a mass basis by Folin–Denis method (Gahler et al., 2003) using gallic acid as a standard. The extension units were determined to be epicatechin, epigallocatechin, (epi) galgallocatechin-3-O-gallate, and (epi) catechin-3-O-gallate with the relative moles of 1.21, 0.31, 13.33 and 9.01, respectively, and the terminal units were determined to be catechin, (epi) galgallocatechin-3-O-gallate, and myricetin with the relative moles of 0.26, 0.52 and 0.22, separately. The mean degree of polymerization was 23.7 by HPLC–MS–MS analysis (Xu et al., 2012). The proposed structure of persimmon tannin was as we previously reported (Yang et al., 2012). BSA was labeled using Remazol brilliant blue dye according to the method of Asquith and Butler (Asquith and Butler, 1985). The protein was dialyzed (12000 MWCO) against acetate buffer for 48 h at 4 °C.

All working solutions were prepared in 0.01 M Tris–HCl buffer solution (pH 7.4). All chemicals were of analytical reagent grade, and doubly distilled water was used in all the experiments.

2.2. Methods

2.2.1. Phospholipase A2 (PLA2) activity

PLA2 enzymatic activity was determined according to the methods of Kawauuchi et al. (1971), using hen’s egg yolk emulsion (prepared by mixing freshly separated egg-yolk with one volume of 8.5% NaCl as the substrate). Released free fatty acids were titrated with KOH (0.02 M) using a pH meter. PLA2 activity was expressed in μEq KOH/min/mg protein under the conditions of the method (mean ± SD, n = 4).

2.2.2. Protein–PT40 binding assay

The reaction mixture (400 μL) contained 100 μg of dye-labeled BSA, 20 μg of PT40 and 10–24 μg of unlabeled BSA or 10–24 μg of PLA2. Each point was replicated 3 times. The amount of competitor required to cause 50% inhibition of the precipitation of tracer protein (I50) (Hagerman and Butler, 1981), was used to compare the relative affinity of PT40 for PLA2 and BSA. Data were fit and statistically analyzed using GraphPad Prism 4.0.

We assessed the ability of PT40 to inhibit PLA2 even in the presence of BSA using activity assays. We mixed 250 μg of native BSA with 50 μg of PT40 in 400 μL of Tris–HCl buffer (pH 7.4), incubated the mixture at 37 °C for 10 min, and
and BSA to PT40 was 1:1. The FT-IR spectra of free PLA2 or enzyme activity of PLA2 in the supernatant was measured. 30 min before centrifuging at 5000

Different lower case letters indicate a statistically significant difference between PT40 for a single protein (p < 0.05). The range of values indicates the 95% confidence limit for the I50.

Table 1
Competitive binding assays for BSA and PLA2.

<table>
<thead>
<tr>
<th>pH</th>
<th>PT40 (µg)</th>
<th>I50 (µg competing protein)</th>
</tr>
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<tbody>
<tr>
<td>7.4</td>
<td>20</td>
<td>246.5, 240.3–251.5, 187.6–198.5</td>
</tr>
</tbody>
</table>

2.2.3. FT-IR difference spectra

FT-IR difference spectra were collected at room temperature on a Nicolet Nexus 670 FT-IR Spectrometer (USA) equipped with a germanium attenuated total reflection (ATR) accessory. All spectra were taken via the attenuated total reflection (ATR) method with a resolution of 4 cm⁻¹ and 60 scans. The infrared spectra of free protein or protein–PT40 complex were obtained between 1800 and 1500 cm⁻¹. For FT-IR studies the concentrations of PLA2 and BSA were fixed at 2.0 µM. The molar ratios of PLA2 to PT40 was 2.5:1, and BSA to PT40 was 1:1. The FT-IR spectra of free PLA2 or BSA were acquired by subtracting the spectrum of the Tris–HCl buffer solution from the spectrum of the protein solution, and the difference spectra of PLA2 or BSA were obtained by subtracting the spectrum of PT40-free protein from that of PT40–PLA2 or BSA–PT40 at the same concentration.

2.2.4. CD spectra

CD measurements were carried out on a J-810 automatic recording spectrophotometer (Jasco Corp., Japan) at room temperature. The spectra were recorded in the range of 195–250 nm with a scan rate of 100 nm/min and a response time of 1 s. For CD studies the concentrations of PLA2 and BSA were fixed at 6.0 µM and 2.0 µM, respectively. Two molar ratios of PLA2 to PT40 (1.7:1 and 2.5:1), and BSA to PT40 (1:1 and 1:1) were used. After each mixture was centrifuged at 4000 × g for 5 min, it was transferred to a 1 mm path-length quartz cuvette. Three scans were accumulated for each spectrum and all the spectra were corrected by subtraction of spectra of buffer of free tannin.

2.2.5. RLS spectra

RLS spectra were recorded by scanning both the excitation and emission monochromators of a spectrophotofluorometer with ∆λ = 0 nm. Various concentrations of PT40 (0–4.0 µM) were mixed with 2.0 µM of PLA2 or BSA in Tris–HCl buffer solution (pH 7.4). RLS spectra were obtained by synchronous scanning on a FP-6500 spectrofluorophotometer (Jasco Corp., Japan) with the wavelength range of 300–600 nm with slit widths of 3 nm for both excitation and emission. The operations were carried out at room temperature.

2.2.6. Chemical modification of PLA2

Chemical modification of His and Lys residues of PLA2 was performed with p-bromophenacyl bromide (p-BPB, Sigma, St. Louis, MO) or acetic anhydride as described by Diaz-Oreiro and Gutierrez (1997). Excess reagent was removed by dialysis against Tris buffer (pH 7.4) at 4 °C for 48 h.

2.2.7. Determination of PT40 precipitated by protein

To determine the amount of PT40 precipitated by BSA or native or modified PLA2, various amounts of protein were mixed with PT40. The mixtures were vortexed vigorously, incubated for 30 min, and centrifuged at 4 °C for 5 min at 5000 × g. The supernatants were removed by aspiration, and the precipitates were gently washed with 100 µL of buffer before centrifuging again for 1 min. Each precipitate was

![Fig. 1](https://example.com/fig1.png)

Fig. 1. Relative PLA2 activity in the supernatant with increasing concentration of PLA2 in the presence of 50 µg PT40 and 250 µg BSA at pH 7.4.
dissolved in 100 μL of sodium dodecyl sulfate/triethanolamine (SDS/TEA) solution and reacted with 50 μL of 0.01 M FeCl₃. After 15 min, the absorbance at 510 nm was recorded (Hagerman and Butler, 1978). The amount of PT40 precipitated was obtained from a standard curve for the color yield of PT40 with the iron chloride in the SDS/TEA solution.

2.2.8. SDS-PAGE and redox-cycling staining

PLA₂ and PT40 (molar ratio of 2.5:1) were mixed and incubated at 37 °C for 40 min before centrifuging for 5 min at 5000 × g. The supernatants were discarded and the precipitates were each washed with 100 μL of buffer three times. The precipitate was dissolved in 100 μL of SDS/TEA

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**Fig. 2.** Influence of increasing amount of BSA on PLA₂ activity in the presence of 50 μg PT40 at pH 7.4.

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**Fig. 3.** FT-ATR-IR spectra of PLA₂ and BSA in aqueous solution. (a) FT-IR spectrum of PLA₂; (b) FT-IR difference spectrum of PLA₂ obtained by subtracting the spectrum of the PT40-free form from that of the PT40-bound form in the region of 1800–1500 cm⁻¹ at pH 7.4 ([PLA₂] = 2.0 μM; [PT40] = 0.9 μM) (c) FT-IR spectrum of BSA; (d) FT-IR difference spectrum of BSA obtained by subtraction the spectrum of the tannin-free form from that of the tannin-bound form in the region of 1800–1500 cm⁻¹ at pH 7.4 ([BSA] = 2.0 μM; [PT40] = 0.9 μM).
solution and 20 μL of the solution was subjected to SDS-PAGE according to the method of Laemmli (1970) with a 15% acrylamide separating gel and a 5% stacking gel. Gels were stained with Coomassie Brilliant Blue G-250. For redox-cycling staining, gels were blotted onto PVDF membranes by semidry blotting, and the PT40-modified proteins were detected with nitroblue tetrazolium (NBT) and glycinate as described by Akagawa et al. (2006). After washing the membrane with distilled water, it was stained with 0.24 mM NBT in 2 M potassium glycinate (pH 10) for 15 min in the dark and then washed thoroughly with distilled water.

3. Results and discussion

The dye-labeled BSA assay is a convenient way to compare the relative affinity of tannins for various proteins at a range of pH values including physiological pH (7.4) (Asquith and Butler, 1985). Although precipitation of BSA by many tannins is maximized at pH 4–5 with little precipitation at higher or lower pH values, in our preliminary studies, we found BSA could precipitate more than 85% of the PT40 in the range of pH 3.0–8.0 with the maximum precipitation of 95% of the tannin at pH 4.0 (determined by SDS-TEA/FeCl3 method). For PLA2, more than 71% of PT40 precipitation of 95% of the tannin at pH 4.0 (determined by SDS-TEA/FeCl3 method). For PLA2, more than 71% of PT40 was precipitated in the pH range between 3 and 8, with 96% of the tannin precipitated at pH 5.0.

Binding affinities of polyphenols for proteins can be described as I50 values, which represent the amount of competitor required to inhibit precipitation of a labeled binding agent by 50% (Hofmann et al., 2006). In general, the higher I50 value, the lower the relative affinity. First we compared the relative affinity of PT40 for native BSA as a competitor against 100 μg of the tracer protein, blue-dye labeled BSA. It took 247 ± 5 μg of native BSA to inhibit precipitation of the dye-labeled BSA by 50%, indicating that the PT40 had a 2.5-fold lower affinity for the native BSA than for the labeled BSA. The I50 value for PLA2 was 193 ± 6 μg, indicating that PT40 has 2-fold lower affinity for the toxin protein than for the tracer protein. However, PT40 has a higher affinity for PLA2 than for BSA at pH 7.4 (p < 0.05) (Table 1).

We carried out direct competition experiments in which we changed the order of addition of PLA2 and BSA in order to evaluate their relative binding to PT40. In our preliminary experiments, we observed that when 250 μg of BSA was mixed with 50 μg of PT40 at 37 °C and pH 7.4 for 10 min, 93% of the PT40 was precipitated by the BSA, which meant that only 3.6 μg of the PT40 was free under the experimental conditions. Surprisingly, this BSA-PT40 mixture was an effective inhibitor of PLA2 even though very little of the PT40 appeared to be available to bind PLA2. When large amounts of PLA2 were added to the BSA-PT40 mix, a relatively small amount of the expected PLA2 activity was detected (Fig. 1). This suggests that PLA2 was inhibited by BSA-bound PT40, or that PLA2 had a higher affinity for PT40 than BSA and replaced PT40-bound BSA. In either case, PLA2 would be inhibited by PT40 even in the presence of an excess of BSA. In the same experiments, there was almost no PLA2 activity in the precipitates.

To further evaluate the relative affinity of PT40 for PLA2, we used PLA2 as the first binding agent and then added increasing amounts of BSA to the mixture as a competitor. As shown in Fig. 2, the relative activity of PLA2 in supernatant did not change as BSA was added. Even the largest amount of added BSA did not reverse the PT40 derived inhibition of PLA2, suggesting that PT40 has much higher affinity for PLA2 than for BSA. Alternatively, PT40 may form more stable complexes with PLA2 than with BSA. Again, the activity of PLA2 in precipitates was almost completely and irreversibly inactivated.

We used FT-IR spectroscopy to establish whether there were conformational changes when PT40 bound to PLA2 or BSA. Infrared spectra of proteins display many amide bands, which represents different vibrations of the peptide moiety. Among these amide bands of the protein, the amide I and II bands in the range of 1600–1700 cm⁻¹ (mainly C=O stretch) and the amide II band near 1548 cm⁻¹ (C–N stretch coupled with N–H bending mode) have been widely used in studies of the secondary structure of the protein (Surewicz et al., 1993). In our study, we found that the optimal binding of BSA or PLA2 with PT40 was reached at mass ratio of 5.28:1 and 2.48:1, respectively, which are 1:1

**Fig. 4.** CD spectra of PT40-PLA2 and PT40-BSA complexes at pH 7.40; T = 298 K: A: PT40–PLA2 complexes (a) 6.0 μM PLA2; (b) 6.0 μM PLA2 + 2.25 μM PT40; (c) 6.0 μM PLA2 + 24.4 μM PT40; B: PT40–BSA complexes (a) 2.0 μM BSA; (b) 2.0 μM BSA + 1.8 μM PT40; (c) 2.0 μM BSA + 2.0 μM PT40.
and 2.5:1 at molar ratios. We choose these ratios of protein to PT40 to study the conformational changes of PLA2 or BSA molecules in the presence of PT40. Fig. 3 shows the FT-IR spectra of the free PLA2 or BSA and the difference spectrum after adding PT40. As shown in Fig. 3, free PLA2 and BSA had amide I peaks at 1658.69 cm\(^{-1}\) or 1645.19 cm\(^{-1}\) respectively and amide II bands at 1541.04 cm\(^{-1}\) and 1546.82 cm\(^{-1}\). Upon interacting with PT40, the amide I peak of PLA2 demonstrated a significant blue shift from 1658.69 cm\(^{-1}\) to 1633.62 cm\(^{-1}\), and a 30 cm\(^{-1}\) great red shift (from 1541.04 to 1571.90 cm\(^{-1}\)) in the amide II band. However, binding PT40 caused only a very small red shift in the amide band I (5 cm\(^{-1}\)) and band II (2 cm\(^{-1}\)) of BSA. Greater spectral shifts for PLA2 after it bound to PT40 than for BSA suggested that PT40 affected the secondary structures of PLA2 more severely than it affected BSA (Wang et al., 2010).

CD can be used to monitor secondary structure changes in proteins. We used CD to evaluate the secondary structure changes that take place when PLA2 or BSA bind PT40. In our preliminary study, we found that the optimal binding was reached at molar ratio of about 2.5:1 for PLA2/PT40 and 1:1 for BSA/PT40. So we choose two molar ratios of PLA2 to PT40 (2.7:1 and 2.5:1), and BSA to PT40 (1.1:1 and 1:1) for more detailed study. As shown in Fig. 4, the addition of PT40 changed the CD spectra of PLA2 and BSA. The CD spectrum of PLA2 displayed two negative bands at 207 and 223 nm, and that of BSA has bands at 208 and 222 nm. These bands, in the region of 195–250 nm, suggest the predominant presence of \(\alpha\)-helical structure (Shen et al., 2007). The band intensities of BSA at 208 and 222 nm decreased with a negative cotton effect through the binding of PT40, but there was no shift in the peaks, clearly indicating that PT40 induced limited secondary structure changes in BSA. However, adding PT40 to PLA2 decreased the intensity of these bands accompanied by an obvious red shift in both bands, indicating considerable changes in the protein secondary structure with the reduction of the \(\alpha\)-helical content in the PLA2.

The RLS spectra of PT40–PLA2 in Tris–HCl buffer solution (pH 7.4) are shown in Fig. 5. PT40 or PLA2 alone showed rather weak RLS intensities. Upon adding a trace amount of PT40 to PLA2 solution, a remarkably enhanced RLS with a maximum peak at 420 nm and a secondary one at 490 nm was observed, exhibiting a PT40 concentration dependent relationship. The enhanced RLS signals were due to the complex formation between PT40 and PLA2 in solution. Similar changes were noted when a trace amount of PT40...
was added to BSA solution. BSA alone showed very low RLS intensities, while BSA–PT40 solution showed notably higher RLS with a maximum peak at 425 nm and a weaker one at 510 nm. The production of RLS is correlated with the formation of aggregates (Xiao et al., 2007). Moreover, other factors including the increase of molecular volume and hydrophobicity, resonance energy transfer between absorption and scattering, as well as the conformational changes of the protein contribute to the RLS enhancement (Zohoorian-Abbootorabi et al., 2012). From the results of FT-IR and CD, we could clearly see that, PT40 induced great conformational changes of PLA2.

To compare the ability of PT40 to form aggregates with PLA2 and BSA, compared the RLS before and after centrifugation at 5000 g for 15 min. As seen from Fig. 5, the RLS intensity of PT40–BSA system hardly changed before and after centrifugation, however, the RLS intensity of PT40–PLA2 system decreased significantly after the centrifugation. Fig. 6 showed the plots of the RLS intensity at the maximum peak against the ratio of [PT40]/[PLA2] or [PT40]/[BSA] before and after the centrifugation. As seen from Fig. 6, the plot of PT40–BSA system before and after centrifugation was the same, demonstrating PT40 did not form aggregates on PT40–BSA system. However, the RLS intensity at 420 nm of PT40–PLA2 system decreased markedly after centrifugation. When the molar ratio of PT40 to PLA2 exceeded 0.45:1, the plot became flat, indicating that the aggregate had formed and the further cross-linking between the aggregates might occur. From these results, we concluded that under the current experiment conditions, the critical induced aggregation concentration value of PT40 on the PT40–PLA2 system was about 0.675 μM, but even at the concentration of 4.0 μM, PT40 still could not induce the aggregates on the PT40–BSA system. It is important to note that, smaller critical induced aggregation concentration value assured a higher affinity to create aggregates and a greater interaction between drugs and proteins (Melo and Ownby, 1999). Therefore, the RLS results indicated that PT40 has higher relative affinity for PLA2 than for BSA under the current experiment conditions, consistent with the results of dye-labeled BSA assay and the competitive binding assay.

Structure–function relationships of PLA2 revealed that His 48 is highly conserved in snake venom PLA2, and participates in catalysis, as well as the toxic and pharmacological effects of PLA2 (Melo and Ownby, 1999). In addition, lysine, cysteine, methionine, tyrosine and tryptophan residues also play very important roles in the myotoxic, edema-inducing, and lethal activities of snake venom PLA2 (Soares and Giglio, 2003). Our previous studies indicated that PT40 was a strong non-reversible inhibitor of the catalytic activity and edema-inducing activity and lethality of Chinese cobra PLA2 (Xu et al., 2012). The current study demonstrates PT40 has a high affinity for PLA2. Amino acid analysis showed that similar with PLA2 from other sources the PLA2 we used here contains 9 lysine residues, 1 histidine residue (Data not shown) (Zhou et al., 2004). Lysine residues are amines, which could covalently react easily with quinones (Kroll et al., 2000). Therefore, we modified the His residues and Lys residues of PLA2 by p-BPB, acetic anhydride, and compared the binding capability of the PLA2 to PT40 before and after the modification to confirm if His or Lys residues were directly involved in the binding sites. As shown in Fig. 7, the binding capability of the PLA2 to PT40 changed slightly before and after the p-BPB modification. However, after the Lys residues was modified, the binding capability of the PLA2 to PT40 declined significantly at all the tested ratios, indicating that Lys residues might be one of the binding sites of PLA2 to PT40.

We further examined the complex formed by PLA2 (modified or unmodified) with PT40 by SDS-PAGE/electroblotting followed by redox-cycling staining with NBT/glycinate. At a basic pH, the catechol moiety can catalyze redox cycling in the existence of glycinate, producing superoxide that reduces NBT to the blue–purple insoluble formazan on the membrane, permitting the detection of phenol-bound protein (Ishii et al., 2008). Unmodified protein or p-BPB-modified protein plus PT40 yielded Coomassie blue- and NBT-stained complexes that were poorly resolved on the gel, but acetic anhydride-modified PLA2 plus PT40 did not yield visible complexes with either staining system (data not shown). TNBS analysis showed that 61% of the free amines were blocked by acetic anhydride. These data provide additional support for the hypothesis that PT40 react is with PLA via reaction with lysine but not histidine.

Fig. 6. The plots of the RLS intensity at the maximum peak against the ratio of [PT40]/[PLA2] or [PT40]/[BSA] before and after the centrifugation. A: PT40–PLA2 complex; B: PT40–BSA complex (■ Before; ● After).
Some phenolic compounds such as flavonoids inhibit the enzymatic capacity of isolated PLA2 and consequently decrease the pharmacological activities such as inflammatory and edema-inducing effects (Fawzy et al., 1988). Tichi et al. (2005) reported that a phenolic compound, rosmarictory and edema-inducing effects (Fawzy et al., 1988). Tichi decrease the pharmacological activities such as in by the basic PLA2, but caused no signi-ificant change in the target PLA2. Reports from Iglesias et al. (2005) suggested that the flavonoid morin decreased the enzymatic and antibacterial activities of snake venom PLA2, and speculated that the mechanism might involve the oxidation of some amino acid residues of PLA2. Our results suggested the PT40 significantly and irreversibly inhibited the catalytic activity of Chinese cobra PLA2, as well as its edema-inducing activity and lethality. In addition, PT40 caused significant conformational changes in the target PLA2 by covalent binding to the protein.

In summary, the binding characteristics of a polymeric persimmon proanthocyanidin fraction PT40 with Chinese cobra PLA2 and BSA were compared by dye-labeled BSA competitive binding assay, by activity assays, by FT-IR, CD and by RLS methods. The results revealed that PT40 has a higher affinity for PLA2 than for BSA at physiological pH. It induced greater conformational changes in PLA2 than that in BSA. In addition, it covalently bound to PLA2 and Lys residues. We propose that the high affinity of PT40 for PLA2 and covalent modification of PLA2 by PT40 may be responsible for the ability of PT40 to irreversibly inhibit PLA2 catalytic activity and edema-inducing activity. PT40 may be an effective antidote to the lethality of Chinese cobra PLA2 in vivo.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 31271833), Special Fund for Agro-scientific Research in the Public Interest (No. 2012030347), and Fundamental Research Funds for the Central Universities (2012PY003).

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

Fawzy, A.A., Vishwanath, R.S., Franson, R.C., 1988. Non-pancreatic phospholipases A2 by retinoids and lysine-arginine residues. We propose that the high affinity of PT40 for PLA2 and covalent modification of PLA2 by PT40 may be responsible for the ability of PT40 to irreversibly inhibit PLA2 catalytic activity and edema-inducing activity. PT40 may be an effective antidote to the lethality of Chinese cobra PLA2 in vivo.

Fig. 7. The binding capability of the PLA2 to PT40 before and after the modification of PLA2 by acetic anhydride and p-BPB separately. A: modification by acetic anhydride; B: modification by p-BPB.


