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Binding sites for interaction of peroxiredoxin 6 with surfactant protein A

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Abstract

Peroxiredoxin 6 (Prdx6) is a bifunctional enzyme with peroxidase and phospholipase A₂ (PLA₂) activities. This protein participates in the degradation and remodeling of internalized dipalmitoylphosphatidylcholine (DPPC), the major phospholipid component of lung surfactant. We have shown previously that the PLA₂ activity of Prdx6 is inhibited by the lung surfactant-associated protein called surfactant protein A (SP-A) through direct protein-protein interaction. Docking of SPA and Prdx6 was modeled using the ZDOCK (zlab.bu.edu) program in order to predict molecular sites for binding of the two proteins. The predicted peptide sequences were evaluated for binding to the opposite protein using isothermal titration calorimetry and circular dichroism measurement followed by determination of the effect of the SP-A peptide on the PLA₂ activity of Prdx6. The sequences ¹⁹⁵EEEAKKLFPK₂₀₄ in the Prdx6 helix and ⁸³DEELQTELYEIKHQIL₉₉ in SP-A were identified as the sites for hydrophobic interaction and H⁺-bonding between the 2 proteins. Treatment of mouse endothelial cells with the SP-A peptide inhibited their recovery from lipid peroxidation associated with oxidative stress indicating inhibition of Prdx6 activity by the peptide in the intact cell.

Keywords

phospholipase A₂; lung surfactant; isothermal titration calorimetry; circular dichroism; lung lamellar bodies

INTRODUCTION

Peroxiredoxin 6 (Prdx 6) is expressed widely in tissues but is especially enriched in lung where it is present in alveolar type II cells, alveolar macrophages, bronchiolar epithelium, and endothelial cells (7, 17, 26). Subcellular fractionation of lungs and cells has indicated the presence of Prdx6 in the lamellar body, lysosomal, and cytosolic fractions (1) and we

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have identified a specific amino acid sequence that is required for organellar targeting of Prdx6 (27, 28). Lung lamellar bodies are lysosomal related organelles (LROs) that, similar to lysosomes, maintain an acidic internal pH that is necessary for basal PLA₂ activity of the enzyme (3).

The latter activity has been called aiPLA₂ as it is measured *in vitro* under acidic, calcium independent conditions (1, 17, 18). The important role of Prdx6 in the metabolism of lung dipalmitoylphosphatidylcholine (DPPC), the lipid component of the lung surfactant that is primarily responsible for its biological activity, has been demonstrated by the study of mice with ‘knock-out’ or overexpression of Prdx6 (13, 14) and by use of a competitive transition state analog inhibitor of aiPLA₂ activity (MJ33) in intact rats, isolated perfused rat lungs, isolated rat alveolar epithelial type II cells, and isolated lung lamellar bodies (8, 9).

Lung surfactant protein A (SP-A) is the major specific protein associated with the lung surfactant (29). Our initial studies of SP-A interaction with PLA₂ proteins demonstrated its binding to Habu snake venom PLA₂ and inhibition of its enzymatic activity; SP-A did not bind to or inhibit activity of several other snake venoms that were tested concurrently (11). Subsequently, we showed specific inhibition of lung aiPLA₂ activity by SP-A (10); the protein expressing this PLA₂ activity was later identified as Prdx6 (5, 17, 18). Addition of SP-A to rat lung homogenate, isolated lamellar bodies, or isolated rat alveolar type II cells inhibited both aiPLA₂ activity and the degradation of DPPC (10) while SP-A “knock-out” resulted in increased lung aiPLA₂ activity (16). Thus, aiPLA₂ activity can be modulated by the presence of SP-A. This inhibiting effect of SP-A on the aiPLA₂ activity of Prdx6 occurs through direct protein-protein interaction (32). Since SP-A and Prdx6 are present in the same acidic compartments of the lung (the LROs), their interaction could be important in the regulation of aiPLA₂ activity in those organelles with a consequent effect on lung phospholipid metabolism.

The present study was designed to determine the molecular sites for interaction of the 2 proteins. We first determined potential interaction sites between SP-A and Prdx6 using the Z-DOCK program and then evaluated these theoretical sites for their interaction with the native protein using synthetic peptides based on the *in silico* analysis.

MATERIALS AND METHODS

Materials

1,2-dipalmitoyl, *sn*-glycerol-3-phosphocholine (DPPC), egg phosphatidylcholine (PC), phosphatidylglycerol (PG), and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL). 1-palmitoyl, 2-[9, 10 -³H]-palmitoyl, *sn*-glycero-3-phosphocholine (³H – DPPC) was obtained from American Radiolabeled Chemicals (St. Louis, MO). Triton X-100 was obtained from Roche Diagnostics (Indianapolis, IN), NaCl, Mg Cl₂, and ATP from Fisher Scientific (Pittsburgh, PA), EDTA from GIBCO (Grand Island, NY), DMEM with isopropyl β-D-1-thiogalactopyranoside (IPTG) from Denville Scientific (Metuchen, NJ), extracellular-signal regulated kinase 2 (Erk 2) from Upstate (Millipore, Billerica, MA), Chariot™ protein delivery reagent from ActiveMotif (Carlsbad, CA), and His-Bind resin from Novagen (EMD, San Diego, CA). A549 lung epithelial cells (CCL-185), a human lung

carcinoma cell line, were obtained from the American Type Culture Collection (Manassas, VA). Pulmonary microvascular endothelial cells (PMVEC) that were isolated following enzymatic digestion of mouse lungs are maintained in our laboratory (22, 23); cells were used at passages 8–13.

Design of peptides

SP-A and Prdx6 peptides were designed with Protein docking software (<http://zlab.bu.edu/zdock>) using structures/sequences of SP-A and Prdx6 that were obtained from the Protein Data Bank. These analyses were based on the published crystal structures of Prdx6 (6) and of a truncated SPA molecule (the carbohydrate recognition domain, CRD) with the site of truncation between the hydrophobic neck region and the collagen-like domain (24). Initial stage docking was modeled using ZDOCK, followed by refinement stage docking and finally clustering of sequences/ structures to predict sequences that might mediate interaction between SPA and Prdx6 proteins (Fig. 1). For further study, we picked the highest scoring sequences that were ₈₃DEELQTELYEIKHQIL₉₉ for the SP-A sequence and ₁₉₅EEEAKKLFPK₂₀₄ for the Prdx6 sequence. A decapeptide corresponding to the proposed Prdx6 sequence and a scrambled peptide using the same amino acids with one extra Ala (PAEKLKAFEKE) were synthesized by GenScript (Piscataway, NJ). A 16 amino acid SPA peptide corresponding to the SP-A sequence and a scrambled peptide using the same amino acids (LELDEEITEYQKQLHI) were synthesized by Proteintech (Chicago, IL).

Generation and Isolation of Proteins

Recombinant human Prdx6 with a C-terminal His tag was cloned in pET21b and expressed in *E. coli*; his-tagged Prdx6 was purified with a Ni²⁺ column (His-Bind resin) as described previously (5, 19). Prdx6 was phosphorylated *in vitro* by incubation with Erk2, ATP, and MgCl₂ as described previously (25). Human SP-A was isolated by density gradient centrifugation of cell-free bronchoalveolar lavage (BAL) fluid obtained by therapeutic lavage of alveolar proteinosis patients at the Hospital of the University of Pennsylvania as described previously (2). SP-A was extracted from whole surfactant using 1-butanol and β-D-glucopyranoside (15, 30).

Human Prdx6 cDNA, cloned into pET21b plasmid, was the starting plasmid for production of Prdx6 deletion proteins (5). Deletions of aa 210–225 (Prdx6 210–225) and aa 195–225 (Prdx6 195–225) were introduced by PCR. The forward primer for both deletions was: 5'-ATGCCATATGCCCGGAGGTCTGCTTCTCG-3'. This primer has an NdeI site (underlined) containing the start codon (bolded). The reverse primer for generating Prdx6 195–225 had the sequence: 5'-ATCGCTCGAGAGGGATGGTTGGAAGGACCATCAC-3'. This primer contains an XhoI site (underlined) and the last codon of the coding region (bolded). The PCR product contains amino acids 1–194; thus, this construct removed the putative SP-A binding region (amino acids 195 to 204) as well as the remainder of the carboxy terminus of Prdx6. The reverse primer for the 210–225 deletion had the sequence: 5'-ATCGCTCGAGTTTGGTGAAGACTCCTTTTCGG-3'; it also contains an XhoI site (underlined). The PCR product that was obtained (Prdx6 210–225) was depleted of the carboxy terminus region but retained the putative binding site and was used as a positive

control for binding. The PCR products for each deletion were cleaved with NdeI and XhoI and cloned into the original vector from which the full-length Prdx6 had been removed by cleavage with the same enzymes. This cloning strategy allowed an in-frame fusion with the His-tag and production of the two His-tagged proteins in which different lengths of the carboxy terminus had been deleted. Deletion constructs were purified on a Ni²⁺ column as described above for recombinant human Prdx6.

Rationale for choice of buffer

SP-A is a secreted protein that is stored prior to secretion in the lung lamellar bodies. Prdx6 is a cytoplasmic protein that also localizes to lamellar bodies through a specific transport mechanism (27, 28). As the lamellar bodies (or possibly lysosomes) are the only known cellular site for co-localization of these two proteins, we reasoned that this compartment would be the likely site of physiologically relevant interaction. Since the luminal pH of lamellar bodies is acidic (3), similar to lysosomes, we carried out our studies of protein/peptide interaction at pH 4.

Isothermal titration calorimetry (ITC)

ITC titrations were performed using a VP-ITC 200 Micro Calorimeter (Micro Cal, Northampton, MA) to determine interaction of the proteins/peptides. Samples were degassed for 5 min with gentle stirring under vacuum. The reaction cell was filled with 11 μ M recombinant Prdx6 or native SP-A and the injection syringe was filled with either 200 μ M of SP-A peptide, Prdx6 peptide, scrambled peptide, or truncated Prdx6 protein. Titration was performed at 25°C in 50 mM acetate buffer at pH 4. Each experiment consisted of 20 injections, 2 μ l each, at intervals of 4 min with continuous stirring at 500 rpm. The corresponding heat of dilution of protein/peptide samples that were titrated into the buffer was subtracted as the background value. Binding stoichiometry (n) and thermodynamic parameters (enthalpy and entropy changes) were determined by using the One Set of Sites Model in the Micro Cal Origin 5.0 program supplied with the instrument.

Circular Dichroism (CD)

CD spectra were recorded in 50 mM acetate buffer (pH 4) in a fused quartz cell with a path length of 0.1 cm using AVIV 202 and 62 DS CD spectrometers (AVIV, Lakewood, NJ) equipped with a thermoelectric cell holder. Temperature was maintained at 25°C using a Pelletier element. Proteins or peptides (50 μ M) were incubated for 15–20 min at 25°C before measurements. Spectra were recorded with three repeats in the far-ultraviolet region (190–260 nm) with a bandwidth of 1.0 nm, a step size of 1 nm, and an integration time of 30s. The buffer baseline was subtracted.

Phospholipase A₂ assay

PLA₂ activity was measured using mixed unilamellar liposomes as substrate as described previously (10). Liposomes consisting of ³H-DPPC, egg phosphatidylcholine, phosphatidylglycerol, and cholesterol (0.5:0.25:0.1:0.15, mol fraction) were generated by extrusion through a 100 μ m pore size membrane under pressure. Substrate was incubated with Prdx6 $\pm$ peptide (50 mg) in 1 ml Ca⁺⁺ – free buffer (40 mM Na acetate, 5 mM EDTA)

at pH 4 for 1h. The radiolabeled free fatty acid product was extracted, resolved by thin layer chromatography using hexane/ether/acetic acid, and analyzed by scintillation counting.

Cell culture and SP-A peptide delivery

A549 cells were grown in DMEM supplemented with 10% fetal bovine serum and antibiotics in 5% CO₂ in air at 37°C. PMVEC were cultured similarly with Plasmocin (InvivoGen, San Diego, CA) added to the culture medium. SP-A peptide or SP-A scrambled peptide was delivered to cells that were at ~50% confluence in six-well plates (0.5 µg peptide in 6 µl Chariot™ protein delivery reagent per well) according to the manufacturer's protocol. Cells were homogenized at 4 h (A549) or at 1 h and 24 h (PMVEC) after protein delivery for measurement of PLA₂ activity.

Recovery of cells from oxidant stress

We have recently shown that the PLA₂ activity of Prdx6 is required by endothelial cells for their recovery from cell membrane lipid peroxidation associated with oxidant stress (22). We repeated this protocol in the absence and presence of the SP-A peptide in order to evaluate possible inhibition of the PLA₂ activity in intact cells. PMVEC at ~ 90% confluence in 100 mm dishes were incubated with 0.3 mM tertbutylhydroperoxide (t-BOOH) for 4 h (zero time for recovery). The cells were then washed with medium free of t-BOOH and treated with 1 µg SP-A peptide in 60 µl Chariot reagent per plate. Incubation of cells was continued for an additional 4 h. Cells from different wells were analyzed at 0, 1, 2, and 4 h of recovery for their content of thiobarbituric acid reactive substances (TBARS) (22).

Statistical analysis

Values are shown as means ± SE for *n* independent experiments. Statistical significance was assessed with SigmaStat software (Jandel Scientific, San Jose, CA, USA). Group differences were evaluated by 1-way ANOVA followed by Student's *t* test as appropriate. Differences between mean values were considered statistically significant at *P*<0.05.

RESULTS

ITC demonstrated binding between Prdx6 and the SP-A peptide, but not between Prdx6 and the scrambled peptide (Fig 2A,B). Binding between Prdx6 and the SP-A peptide was exothermic at 25°C, allowing calculation of the equilibrium association constant for binding (*K_a*) and the enthalpy (Δ*H*) of association using a single site binding model (Table 1). Data is reported as the dissociation constant (*K_d*=1/*K_a*). Binding also was demonstrated between Prdx6 peptide and SP-A (Fig 3 and Table 1); the latter was measured at pH 4, but the Prdx6 peptide interacted similarly with SP-A in 50 mM phosphate buffer at pH 7 as measured by ITC (data not shown). The *K_d* for binding of the SP-A or Prdx6 peptides to the Prdx6 or SPA proteins, respectively, were in the 1–2 µM range indicating relatively high affinity for the interactions. SP-A bound to truncated Prdx6 210–225 (containing the binding sequence), but there was no interaction with Prdx6 195–225 in which the proposed binding sequence had been removed (Fig 4A,B and Table 1). The measured *K_d* for binding of

Prdx6 210–225 to SP-A was ~1 order of magnitude greater than the K_d for the binding of the Prdx6 peptide to SP-A, but the H and S were similar (Table 1).

The far UV CD spectrum of Prdx6 showed helical structure as indicated by a band of increased absorbance at 200 nm and decreased absorbance at 222 nm and 208 nm. The SP-A peptide alone showed predominantly random coil-pattern with an absorbance peak at 193 nm and a trough at 195 nm (Fig. 5). The SP-A peptide and Prdx6 incubated together showed a change in conformation with an absorbance peak at 193 nm and a trough at 202 nm, significantly different from results for either of the original structures (Fig 5A). The scrambled SP-A peptide had no effect on the CD spectrum of Prdx6 (Fig 5B). Thus, the interaction of Prdx6 with the SP-A peptide resulted in a significant change in its secondary structure.

PLA₂ activity of recombinant human Prdx6 was demonstrated at pH 4 (Table 2). The PLA₂ activity of Prdx6 that had been phosphorylated was markedly increased by about 17-fold (Table 2) as reported previously (31). The calculated turnover numbers (kcat) based on the previously reported data (19) are $2.3 \times 10^5 \text{ sec}^{-1}$ for Prdx6 and $3.8 \times 10^6 \text{ sec}^{-1}$ for phosphorylated Prdx6. The effect of SP-A peptide on PLA₂ activity was evaluated using a molar ratio of ~400 to 1 relative to Prdx6 and ~800 to 1 relative to phosphoPrdx6. Activity was inhibited by ~80% in the presence of SP-A peptide (Table 2), consistent with previous results using full length SP-A (32). Activity of phosphorylated protein was inhibited by ~95% in the presence of SP-A peptide (Table 2). The scrambled SP-A peptide had no effect on PLA₂ activity (Table 2).

We developed an *in vitro* competition assay to evaluate the interaction of the Prdx6 peptide with the SP-A peptide. The SP-A peptide inhibited the PLA₂ activity, as above (Fig. 6). Inhibition of the PLA₂ activity of either Prdx6 or phosphorylated Prdx6 by the SP-A peptide was significantly reversed by the addition of excess Prdx6 peptide; addition of the scrambled Prdx6 peptide had no effect (Fig 6). We interpret these results as indicating that the binding of the SP-A peptide by excess Prdx6 peptide allows the expression of the PLA₂ activity of the full length Prdx6. Thus, these results demonstrate the interaction of the Prdx6 and SP-A peptides.

We evaluated the effect of the SP-A peptide on PLA₂ activity of A549 cells and PMVEC. PLA₂ activity of the homogenate of both cell types was significantly reduced when the SP-A peptide was delivered to intact cells prior to homogenization using the protein delivery reagent; there was no effect on PLA₂ activity with delivery of the scrambled peptide (Table 2). Thus, the SP-A peptide can interact not only with recombinant protein *in vitro* but also with native Prdx6 in both epithelial and endothelial cells. Inhibition was evident at 1 h after delivery of the peptide. PLA₂ activity of the PMVEC had returned to control values when measured at 24 h after peptide delivery (not shown).

We evaluated the effect of the SP-A peptide on the PLA₂ activity of Prdx6 in intact PMVEC by the measurement of TBARS following oxidative stress. PLA₂ activity of Prdx6 is required for the repair of cellular lipid peroxidation in these cells (22). TBARS measured in control cells without exposure to t-BOOH was $1.03 \pm 0.03 \text{ nmol/mg protein}$, mean $\pm$ SE,

n=3 (not shown). There was a 4-fold increase in TBARS after 4 h exposure to t-BOOH; this is called zero recovery time (Fig. 7.). The value for TBARS had returned to the control level by 1h after removal of the oxidant stress in the cells that were not treated with peptide (Fig. 7). The cells treated with peptide showed a markedly delayed recovery and TBARS had still not returned to the control value at 4 h of recovery. These results provide evidence for the inhibition of Prdx6 PLA₂ activity by the SP-A peptide in intact endothelial cells.

DISCUSSION

Our previous studies demonstrated that SPA binds to Prdx6 and inhibits its PLA₂ activity (10, 32). The present study was designed to determine the peptide sequences in Prdx6 and SP-A that are involved in the interaction of the two proteins. To determine the relevant peptides, we designed interacting peptide sequences following analysis of SP-A and Prdx6 proteins using protein docking software. Peptides of SP-A (DEELQTELYEIKHQIL) and Prdx6 (EEEAKKLFPK) were selected based on the *in silico* analysis and evaluated for their importance in SP-A-Prdx6 interaction.

ITC measurements showed relatively high affinity for binding of the synthetic SP-A peptide to Prdx6 and CD spectra indicated that the binding of SP-A peptide to Prdx6 resulted in a change in the secondary structure of the protein. Likewise, ITC analysis confirmed binding of the Prdx6 peptide to SP-A with a dissociation constant similar to that for binding of the SP-A peptide to Prdx6. Truncated Prdx6 proteins that contained the putative binding sequence interacted with SP-A as determined by ITC while no interaction was observed with the protein where the putative binding sequence had been deleted. The negative values for

H and S suggest that the mechanism for binding is through both hydrogen bonds and hydrophobic interactions. Finally, using a competition assay for PLA₂ enzymatic activity, we demonstrated the interaction of the SP-A and Prdx6 peptides. These observations are consistent with the conclusion that the sequences of the two designed peptides are responsible for interaction of the two full-length proteins.

Measurement of PLA₂ activity of recombinant protein, as well as cellular PLA₂ activity, confirmed that the interaction of the SP-A peptide with Prdx6 inhibited its enzymatic function. Change in protein conformation, as shown by CD analysis, presumably accounts for the loss of PLA₂ activity. Although the PLA₂ activity of Prdx6 is involved in cellular phospholipid metabolism, this activity also is required for the activation of NADPH oxidase (NOX2) in pulmonary endothelium, polymorphonuclear leukocytes (PMN), and other cell types (4, 21). Because the generation of reactive oxygen species (ROS) as a result of NOX2 activation is a major cause of tissue injury associated with inflammation, the inhibition of Prdx6 PLA₂ activity could be an important therapeutic modality. Peptide inhibitors for Prdx6 PLA₂ activity have not been described previously. The lipid MJ33 is a known inhibitor of this activity but exerts its effect through occupation of the PLA₂ active site as an analogue of the substrate transition state (9, 12). Knowledge of the SP-A inhibitory sequence could be important for development of a peptide inhibitor of Prdx6 PLA₂ activity, possibly for use as a therapeutic agent for treatment or prevention, of multiple conditions associated with oxidative stress such as acute lung injury (ALI) (20). However, the possible adverse effects must be considered since loss of Prdx6 PLA₂ activity can significantly delay the

resolution of cell membrane lipid peroxidation associated with oxidative stress as shown in the present study and previously (22).

In summary, we have demonstrated the peptide sequences of SPA (DEELQTELYEIKHQIL) and Prdx6 (EEEAKKLFPK) that are responsible for the interaction between these two proteins. The interaction of SPA with Prdx6 can modulate its PLA₂ activity both *in vitro* and *in vivo*.

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HIGHLIGHTS

- The peptides in each protein responsible for the interaction of surfactant protein A (SP-A) and peroxiredoxin 6 (Prdx 6) have been identified
- Binding of the SP-A peptide to Prdx 6 destabilized its secondary structure and inhibited its phospholipase A₂ (PLA₂) activity
- Inhibition of Prdx6 PLA₂ activity by SP-A peptide markedly delayed the recovery of pulmonary microvascular endothelial cells from peroxidative stress

Figure 1. Modeling of the SP-A: Prdx6 interaction with the Z Dock program. (A) Ribbon model of SP-A-Prdx6 complex. Green, blue and purple show the monomers of SP-A; yellow and red show homodimeric Prdx6. (B) Amplified view of interacting α -helices. Yellow-Prdx6 (195EEEAKKLFPPK₂₀₄), blue-SP-A (83DEELQTELYEIKHQIL₉₉).

Figure 2.

ITC for interaction of recombinant Prdx6 with SP-A or SP-A scrambled peptides.

Experiments were performed in 50 mM acetate buffer at pH 4 and 25°C. Horizontal lines in the upper panels are the computer-drawn baselines. Results shown are representative of 3 independent experiments for each condition. (A) Prdx6 titrated with SP-A peptide. (B) Prdx6 titrated with scrambled SP-A peptide. Panels show results after baseline correction.

Figure 3.

ITC for interaction of SP-A with Prdx6 or Prdx6 scrambled peptides. Horizontal lines in the upper panels are the computer-drawn baselines and in the lower left panel is the line of best fit. Results are representative of 3 independent experiments for each condition. Experiments were performed in 50 mM acetate buffer at pH 4 and 25°C. (A) SP-A titrated with Prdx6 peptide. (B) SP-A titrated with scrambled Prdx peptide.

Figure 4.

ITC for interaction of truncated Prdx6 with SP-A proteins. Experiments were performed in 50 mM acetate buffer at pH 4 and 25°C. Horizontal lines in the upper panels are the computer-drawn baselines. Results are representative of 3 independent experiments for each condition. (A) SP-A titrated with Prdx6 210–225. (B) SP-A titrated with Prdx6 195–225.

Figure 5.

Far UV circular dichroism measurements were done in 50 mM acetate buffer at pH 4.

Spectra are the mean of two independent experiments. θ = molecular ellipticity. (A) Spectra for Prdx6, SP-A peptide, and Prdx6+SPA peptide. (B) Spectra for Prdx6, scrambled SP-A peptide, and Prdx6+scrambled peptide.

Figure 6.

Effect of SP-A peptide on PLA₂ activity of Prdx6. PLA₂ activity was measured at pH 4. (A) PLA₂ activity of Prdx6 under control conditions (no additions), in the presence of the SP-A peptide, with both SP-A peptide and excess Prdx6 peptides, or with both SP-A and excess Prdx6 scrambled peptide. (B) Same experiment as (A) but with phosphorylated Prdx6 (pPrdx6). Values are mean $\pm$ SE for n = 3. *P<0.05 vs the preceding bar in (A) or (B).

Figure 7.

Effect of SP-A peptide on recovery of PMVEC from oxidative stress. Cells were treated with 0.3 mM t-BOOH for 4 h. The oxidant was removed and the treated cells were incubated in medium with SP-A peptide with Chariot protein delivery reagent. Cells were analyzed for TBARS at 0, 1, 2, and 4 h after the end of the oxidant exposure.

Table 1

Binding parameters calculated from isothermal titration calorimetry (ITC) using a 1 site binding model.

	N	K _d (μ M)	H (kcal/mol)	S (kcal/mol/deg)
SPA peptide:Prdx6	2.75	1.45	-2.271E4	–
Prdx6 peptide:SPA	2.45	1.52	-1.282E4	-17.11
Prdx6 210–225:SPA	1.06	14.2	-1.466E4	-27.83
Prdx6 195–225:SPA	NB	–	–	–

Values were measured at pH 4 and 25°C. N, stoichiometry (mol/mol); K_d, equilibrium dissociation constant; H, enthalpy; S, entropy; NB, no binding detected.

Table 2Effect of SP-A peptide on PLA₂ activity of Prdx6.

	Prdx6 nmol/min/mg prot	pPrdx6 nmol/min/mg prot	A549 cells nmol/h/mg prot	PMVEC nmol/h/mg prot
No additions (control)	103 ± 0.4	1690 ± 22	3.36 ± 0.11	5.68 ± 0.16
+SP-A peptide	17 ± 0.9*	99 ± 1.9*	1.52 ± 0.10*	1.07 ± 0.05*
+scrambled SP-A peptide	104 ± 1.5	1680 ± 31	3.35 ± 0.06	5.72 ± 0.20

PLA₂ activity of proteins was measured using 80 nM recombinant human Prdx6 or 40 nM phosphorylated Prdx6 (Prdx6) ± 33 µM SP-A peptide. Cells were homogenized at zero time (control) and after 4 h (A549) or 1 h (PMVEC) incubation with SP-A peptide plus Chariot reagent (see Methods). Values are mean ± SE for n=3 for PMVEC and n= 4 for other conditions.

* P < 0.05 vs the corresponding control.