Scientific paper

Cross-affinity of Peptide Ligands Selected from Phage Display Library Against Pancreatic Phospholipase A2 and Ammodytoxin C

Dominik Gaser,[1] Borut Štrukelj,[2] Tomaž Bratkovič,[2] Samo Kreft,[2] Jože Pungerčar[3] and Mojca Lunder[2,*]

[1] *Lek d.d., Verovškova 57, SI-1000 Ljubljana, Slovenia*

[2] *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia*

[3] *Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*

** Corresponding author: E-mail: mojca.lunder@ffa.uni-lj.si; Tel.:+38614769500; Fax: +38614258031*

Received: 10-11-2008

Abstract

Two phage-displayed random peptide libraries were screened for ligands with potential inhibitory activity against pancreatic phospolipase A2 or ammodytoxin C (neurotoxin found in the venom glands of *Vipera ammodytes ammodytes*). The interaction of selected peptides with pancreatic phospholipase A2 and ammodytoxin C was confirmed with surface plasmon resonance and phage ELISA assays. Interestingly, peptides showed equal affinity to both proteins, regardless which of the two proteins was used as a target in the selection procedure. Despite pronounced affinity, none of the synthetic peptides inhibited enzyme targets in *vitro* at the concentrations below 167 μM.

Keywords: Pancreatic phospholipase A2; ammodytoxin C; phage display; peptide ligands

1. Introduction

Pancreatic phospholipase and ammodytoxin C (neurotoxin found in the venom glands of long-nosed viper, *Vipera ammodytes ammodytes*) belong to a group of secretory phospholipases A2 (EC 3.1.1.4) that catalyze the hydrolysis of the *sn*-2 ester bond of phospholipids.

Pancreatic phospholipase is secreted by the pancreas in response to food intake. It is one of the most extensively studied phospholipases in terms of structure and mechanism of action,[1–3] yet its pharmaceutical potential as a drug target has not been fully exploited.[4] Various *in vitro* and *in vivo* studies imply that pancreatic phospholipase hydrolysis of phospholipids in intestinal lumen is required for the efficient absorption of cholesterol.[5–7] In addition, it was shown that hydrolysis of phospholipids on the surface of lipid emulsions is a prerequisite for pancreatic lipase digestion of triglycerides in the core of lipid emulsions, therefore suggesting an important role in fat absorption.[8] Pancreatic phospholipase deficient mice fed high-fat/high-cholesterol diet were resistant to diet-induced obesity and the mechanism for this effect is most likely to be decreased dietary fat absorption.[9]

Inhibiting pancreatic phospholipase and consequently inhibiting nutrient digestion and absorption would reduce energy intake through gastrointestinal mechanism, and therefore represents one strategy by which treatment and/or prevention of obesity might be accomplished.[4]

Secretory phospholipases A2 are also commonly found in snake venoms from *Elapidae* and *Viperidae* families and have been extensively studied due to their pharmacological and pathophysiological effects in living organisms. Several substances have been evaluated regarding their effects against snake venoms and isolated toxins, including plant extracts and compounds from marine animals, mammals and snake serum plasma in addition to poly- or monoclonal antibodies and several synthetic molecules.[10] Research involving snake venom phospholipases may be useful because of their structural homology to

pancreatic and other human phospholipases as well as to provide therapeutic molecular models for antiophidian activity to supplement the conventional serum therapy.

In search for ligands with potential inhibitory activity against phospolipase A2 from porcine pancreas or ammodytoxin C we used two phage-displayed random peptide libraries. Selection of peptides from phage-displayed random combinatorial peptide libraries has proved a successful technique for discovering new ligands of enzymes and other protein targets.[11–13] These peptides are modulators of protein function (inhibitors, agonists, antagonists) and represent a starting point for identifying and synthesizing compounds with peptide characteristics but non-peptide structures, the peptidomimetics (drug leads).[14] Phage-displayed peptide libraries have been successfully used to isolate peptide ligands directed to a functional site for which the natural ligand is not a protein, which is also the case with secretory phospholipases A2.[12–16]

2. Experimental

2. 1. Materials

Two Peptide Library Kits Ph.D.-C7C™ and Ph.D.-12™ were purchased from New England Biolabs (Frankfurt am Main, Germany). Peptides were obtained from Pepscan Presto (Lelystad, Netherlands) and JPT Peptide Technologies GmbH (Berlin, Germany). Ammodytoxin C (EC 3.1.1.4) was purified from *Vipera ammodytes ammodytes* venom as described by Gubenšek et al.[17] The isolated toxin was additionally purified by RP-HPLC using similar conditions as reported by Pungerčar et al.[18] Porcine pancreatic phospholipase A2 (EC 3.1.1.4) was obtained from Boehringer Mannheim (Germany) and appeared as a single Coomasie Brilliant Blue stained band upon SDS-PAGE. Purity of the two phospholipases exceeded 98%. Fatty acid free bovine serum albumin was purchased from Sigma-Aldrich (Steinheim, Germany). The phospholipase substrate used for detection of enzymatic activity was prepared from Pyr-PG (1-hexadecanoyl-2-[1-pyrendecanoyl]-*sn*-glycero-3-phosphoglycerol) purchased from Molecular Probes (USA). 96-well microtitre plates Maxisorp™ were from Nalge Nunc International (Roskilde, Denmark). Horseradish peroxidase-labeled mouse anti-M13 monoclonal antibody was obtained from Amersham Biosciences (Little Chalfont, UK). Active site-directed, specific, competitive, and reversible inhibitor of phospholipases A2, MJ33 (1-hexadecyl-3-trifluoroethylglycero-*sn*-2-phosphomethanol) was from Sigma-Aldrich (Steinheim, Germany). ABTS (2,2'-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid] diammonium salt) and PEG (polyethylene glycol) were also purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals used were of analytical grade.

2. 2. Selection of Phage Clones Binding to Pancreatic Phospholipase A2 or Ammodytoxin C

Pancreatic phospholipase A2 or ammodytoxin C were dissolved in 100 mM $NaHCO_3$ (pH 8.5) to a final concentration of 100 g/ml. Maxisorp surface microtitre plate wells were filled with 100 µl of target enzyme solution and incubated overnight at 4 °C with gentle agitation. Microtitre plates were blocked using 200 µl of 2% fatty acid free bovine serum albumin in phosphate buffered saline (135 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4; pH 7.4) for 1 h at room temperature and rinsed four times with phosphate buffered saline containing 0.1% Tween-20.

An aliquot of 2×10^{11} (Ph.D.-C7C™) or 4×10^{10} (PhD-12™) plaque forming units was diluted to 100 µl with phosphate buffered saline containing 0.1% Tween-20 and incubated in target protein-coated wells for 1 h at room temperature with gentle agitation. Non-binding phages were then discarded by washing the wells 10 times with phosphate buffered saline containing 0.1% Tween-20. Bound clones were eluted nonspecifically with 100 µl of 50 mM Gly-HCl, pH 2.2 for 10 min. With pancreatic phospholipase A2, a separate selection protocol comprising specific elution with 100 µl of 200 µM MJ33 was used.

Low-pH eluate was immediately neutralized with 100 ml of 200 mM phosphate buffer (pH 7.5). Eluates were amplified by infecting *E. coli* ER2738 host cells. After 5 hours of growth at 37 °C bacteria were removed by centrifugation and phages in the supernatant were precipitated by adding 1/6 volume of PEG/NaCl solution (20% PEG-8000, 2.5 M NaCl) and incubated overnight at 4 °C. The precipitate was resuspended in a small volume of phosphate buffered saline and amplified eluates were titered to determine phage concentration. Three additional rounds of biopanning were performed for each selection protocol, increasing the Tween-20 concentration to 0.5% in the washing steps. Finally, eluate from the last round of selection was used to infect plated bacterial host cells and 20–40 resulting plaques were randomly selected. Individual phage clones were then grown and purified for further analysis.

2. 3. Enzyme-linked Immunosorbent Assay (ELISA)

Microtitre plate wells were coated overnight with 100 µl of ammodytoxin C or pancreatic phospholipase solution (50 µg/ml) in 50 mM $NaHCO_3$ (pH 8.5) and blocked with 200 µl of blocking buffer (2% bovine serum albumin in phosphate buffered saline) for 1 h. As negative controls a separate set of wells was blocked with blocking buffer without previous target enzyme immobilization. Plaques from the last round of selection were inoculated in 100-

fold diluted overnight culture of *Escherichia coli* ER2738 and incubated at 37 °C with shaking for 5 h. Next, phages were separated from bacteria by centrifugation. 100 µl of each selected amplified phage clone were diluted to 200 µl with blocking buffer and transferred to target coated and blank wells. Plates were incubated at room temperature for 1.5 h. Wells were then washed 3 times with 0.05% phosphate buffered saline containing 0.1% Tween-20. Horseradish peroxidase-labeled mouse anti-M13 monoclonal antibody in blocking buffer (1:5000) was added (200 µl per well) and incubated for 1 h at room temperature. Finally, wells were washed 4 times with 0.05% phosphate buffered saline containing 0.1% Tween-20. Substrate solution (200 µl of 0.22 mg/ml ABTS in 50 mM citric acid, pH 4.0, 1.7 µl 30% H_2O_2/ml) was added and incubated for 30 min at 37 °C. Absorbance was determined at 405 nm using a Safire microplate reader (Tecan, Austria).

2. 4. Preparation of Phage DNA and Sequencing

Oligonucleotide sequences encoding capsid-displayed peptides were determined for phage clones giving rise to at least four times higher ELISA signals compared to negative controls. Phages were purified by PEG/NaCl precipitation and suspended thoroughly in 100 µl of iodide buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA; 4 M NaI). Phage single-stranded DNA was precipitated with the addition of ethanol. Purified DNA was sequenced with M13 –96 sequencing primer (5'-CCCTCATAGTTAGCGTAACG-3') by MWG Biotech sequencing service (Munich, Germany).

2. 5. Evaluation of Inhibitory Activities of Synthetic Peptides

The phospholipase activities of pancreatic phospholipase and ammodytoxin C in the presence and absence of selected synthetic peptides were measured using the modified method of Radvanyi et al.[19, 20] Ammodytoxin C or pancreatic phospholipase (1.2 nM) were incubated in 50 mM KCl, 1 mM $CaCl_2$, 50 mM Tris/HCl; pH 7.4 buffer, supplemented with 0.06% (w/v) of fatty acid free bovine serum albumin for 30 min with peptides at concentrations 167, 83, 42, 17, 8.3, 4.2 and 1.7 µM. Fluorogenic PyrPG vesicles (1.4 µM final concentration) were used as substrate. Fluorescence was measured in a 96-well plate on Safire microplate reader (Tecan, Austria) over 30 kinetic cycles at 5 s intervals at λ_{ex} 342 nm, λ_{em} 395 nm. Background fluorescence in control experiments lacking the enzyme was recorded and subtracted from the measurements.

2. 6. Surface Plasmon Resonance (SPR) Experiments

SPR experiments were carried out using Biacore T100 optical biosensor (Biacore, Uppsala, Sweden) at 25 °C. Ammodytoxin C or pancreatic phospholipase A2 were dissolved in 10 mM sodium acetate, pH 4 (20 µg/ml), and immobilized onto a CM5 sensor chip using the standard NHS (*N*-hydroxysuccinimide)/EDC (*N*-ethyl-*N*'-[3-dimethyl aminopropyl]-carbodiimide) coupling procedure. Approximately 1000 RU (resonance unit) of ammodytoxin C or pancreatic phospholipase A2 was coupled to the sensor chip surface. Different concentrations of peptides (1, 0.75, 0.5, 0.25, 0.125, 0 mM) were injected over the chip at a flow rate 30 µl/min for one minute in running buffer (phosphate buffered saline containing 0.1% Tween-20, pH 7.4) followed by a dissociation period of one minute. The chip was regenerated by injecting 5 µl of 20 mM Gly-HCl (pH 2.5). The sensorgram signal for each peptide was normalized by subtracting the non-derivatized surface flow-cell signal.

3. Results and Discussion

We used two model secretory phospholipases A2, pancreatic phospholipase isolated from porcine pancreas and ammodytoxin C, presynaptically active nevrotoxin from long-nosed viper, to search for inhibitors of phospholipase activity. Affinity selections were performed with two commercially available phage-displayed peptide libraries. One of the libraries (Ph.D.-C7C™) consisted of constrained (conformationally restricted disulphide-cyclized) peptides. Ligands isolated from such libraries sometimes possess higher affinities to targets, due to the improved entropy of binding, and enable the chance to mimic the discontinuous epitopes.[21] The other library (Ph.D-12™) contained unconstrained (linear) peptides. Phospholipase A2 active site involves hydrophobic channel, for which some linear peptide inhibitors have already been discovered.[22] Affinity of the phage displayed peptides was first determined in ELISA assay. Peptides that showed selective binding to the target were sequenced and are shown in Table 1. Phages displaying constrained peptides indeed showed slightly greater affinity compared to phages displaying linear peptides as judged from ELISA signals (data not shown). Selected peptides show no resemblance to any of the previously reported peptide inhibitors.[23–25]

In ammodytoxin C selection, using constrained library peptide, no clear consensus sequence emerged. However, peptide AT_1 (CWWLHPTHC) predominated, appearing in more than 60% of all phage clones binding to the target. Unconstrained library (Ph.D.-12™) panning resulted in peptides with greater similarity. All selected peptides were rich in Trp, preceded by His. Peptide AT_2 (HWWSPWPNPPQI) appeared on more than 30% of phage binders.

In pancreatic phospholipase selection, a great diversity was observed among selected peptides, even in the case of specific elution with inhibitor MJ33 that should de-

Table 1: Phage clones selected from phage libraries Ph.D.C7C and Ph.D.12 on pancreatic phospholipase A2 or ammodytoxin C. Number in the brackets below each sequence indicates the frequency of phages with the same sequence. Peptides that were chosen for synthesis are depicted in bold font.

Target protein	**Ammodytoxin C**		**Pancreatic phospholipase**			
Elution	**Nonspecific (low pH)**		**Nonspecific (low pH)**		**Specific (MJ 33)**	
Phage library	Ph.D.C7C	Ph.D.12	Ph.D.C7C	Ph.D.12	Ph.D.C7C	Ph.D.12
Displayed peptides	AVCTDF (1)	HWNVWWPVSIPE (1)	NMTQAYS (1)	GPSVGVTASHTR (1)	QVPRSPY (1)	HLWPFYSMPPQH (2)
	HLFTQAF (1)	HSWSFFWQSPAD (1)	NGMYAHP (3)	APWHLSSQYSRT (2)	TTRTMTQ (1)	HPPYWYPWQQSS (1)
	ALFKPSM (1)	HSYWYRWTPSHL (1)	YRQASDS (1)	SNHPATLTGTGG (1)	SMSTTRS (1)	**HGWLYPHPRYPV** (16)
	QTSAMRH (1)	FHWRWSTFPEYP (1)	QLQPTRL (1)	AQPYPFSTRHWQ (1)	LETASNY (1)	
	SLNPSSR (1)	HWRWWQSDHLFT (1)	HSNRLNL (3)	KLWNLHPTQALW (2)	LFHHQAS (2)	
	PRTHYAI (3)	SHWIRYFPWSIG (1)	DHHTYNH (1)		TSAPHRM (1)	
	YFNQPIR (1)	**HWWSPWPNPPQI** (3)	HSPTRGI (1)		TARSPLL (2)	
	LTLNGSP (1)		KDSSLHV (1)		LPSRSHL (1)	
	SSKTSFT (1)				FWQSDKI (1)	
	WWLHPTH (17)					

tach peptide ligands selectively from the active site of phospholipase (and not from other, e.g. allosteric binding sites). Screening of linear peptide library and using a specific elution resulted in predomination of peptide PL (HGWLYPHPRYPV; with more than 80% of all phages binding to the target). His and Trp appeared on the same place as in peptide AT_2 selected on ammodytoxin C. Since His and Trp were also present in the most frequent cyclic peptide selected on ammodytoxin, these three displayed peptides were synthesized (Table 1, peptide sequences depicted in bold). Peptide CSQLQTTKC from selection on pancreatic lipase was used as control.[12] Spacer sequence Gly-Gly-Gly-Ser was added to the C-terminus and the C-terminal carboxylate was amidated to block the negative charge. Phage-displayed peptides are expressed at the N-terminus of pIII phage protein, followed by a short spacer (Gly-Gly-Gly-Ser) and then the wild-type pIII sequence. When designing synthetic peptides corresponding to selected sequences the amidated spacer sequence was added to the C-terminus since free carboxy terminus induces negatively-charged group at a position occupied by a neutral peptide bond during panning, which may hinder binding of synthetic peptide and moreover, adjacent spacer sequence may possibly contribute to the peptide – target interaction.

Constrained peptides (AT_1 and the control peptide) were cyclized by formation of an intramolecular disulphide bond.

The common characteristic of previously reported peptide inhibitors FYSYK[23], LAIYK[24] and WARFS[25] are hydrophobic, aromatic and aliphatic amino acids able to

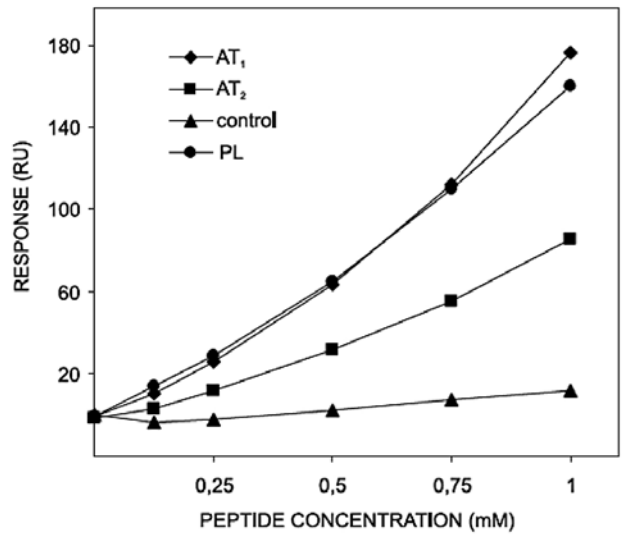

Figure 1: Binding of peptides to pancreatic phospholipase A2. Different concentrations of peptides (1, 0.75, 0.5, 0.25, 0.125, 0 mM) were injected over the immobilized chip. Response reached at different concentrations was measured 5 s before the end of sample injection. Higher concentrations of peptides exhibited higher response (RU), however, RU_{max} was not reached.

provide interactions with active site hydrophobic channel of phospholipases A2.

Since synthesized peptides were also rich in aromatic amino acids, we assumed the binding would be directed towards the hydrophobic channel of the active site and therefore the peptides could directly inhibit enzyme activity.

Affinity to both targets was confirmed by SPR. Figures 1 and 2 demonstrate that peptides AT_1, AT_2 and PL bind to both enzymes, regardless the target used in selections. Best response on both targets was obtained with peptides AT_1 and PL. On the other hand, control peptide showed no affinity to either of the targets.

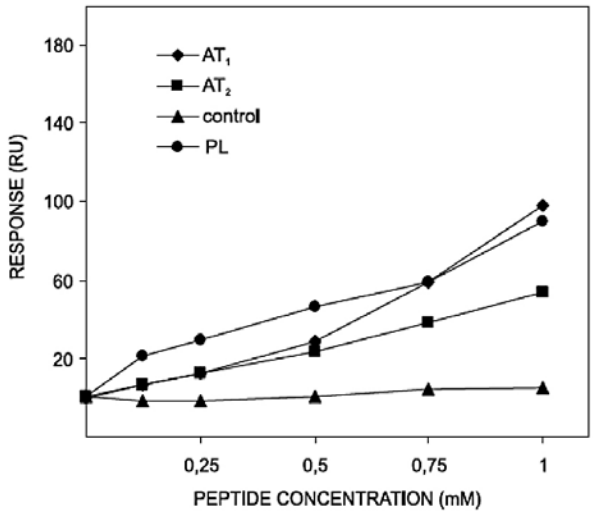

Figure 2: Binding of peptides to ammodytoxin C. Different concentrations of peptides (1, 0.75, 0.5, 0.25, 0.125, 0 mM) were injected over the immobilized chip. Response reached at different concentrations was measured 5 s before the end of sample injection. Higher concentrations of peptides exhibited higher response (RU), however RU_{max} was not reached.

The kinetics of selected peptides is a fast-on and fast-off binding system, therefore kinetic parameters could not be determined. Moreover, the determination of equilibrium dissociation constant (Kd) from Scatchard analysis of the amount of peptide bound at the surface as a function of the concentration of peptide in the buffer was not possible, since applied concentrations of peptide did not reach the maximal response (RU_{max}). Injection of higher peptide concentrations resulted in non-specific binding to the SPR chip.

Ultimately, peptides were tested for their potential inhibitory activity. At the concentration of 167 μM peptides exhibited no inhibition against any of the two enzymes. The peptides AT1 and AT2 were selected by nonspecific elution, therefore they could bind to other sites on the enzyme (not the active site), hence the lack of their inhibitory activity. The peptide PL, obtained by specific elution should theoretically bind to the active site. However, it is possible that the binding of inhibitor MJ33 changed the conformation of target protein, which in turn released a phage bearing PL peptide from a different site. Another explanation could be that peptides bind to the active site, but their affinity is much lower that the affinity of the substrate and inhibition can not be detected at these concentrations of peptides.

Peptides selected in different selection protocols clearly show affinity toward pancreatic phospholipase and ammodytoxin C, regardless of the target used in selection. This proves the homologue structures on the enzyme are responsible for binding of the peptides. However, affinity is low and none of the peptides possess inhibitory activity *in vitro* below 167 μM concentration.

4. Acknowledgements

Surface plasmon resonance experiments were performed in Infrastructural Centre for Surface Plasmon Resonance at the Department of Biology, Biotechnical Faculty, University of Ljubljana. We thank Dr. Zala Jenko Pražnikar for help with the PLA2 activity measurements.

5. Abbreviations

ELISA, enzyme-linked immunosorbent assay; MJ33, 1-hexadecyl-3-trifluoroethylglycero-*sn*-2-phosphomethanol; PyrPG, 1-hexadecanoyl-2-(1-pyrendecanoyl)-*sn*-glycero-3-phosphoglycerol; SPR, surface plasmon resonance; ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt; PEG, polyethylene glycol.

6. References

1. C. Yuan, M. Tsai, *Biochim. Biophys. Acta* **1999**, 1441, 215–222.
2. E. A. Dennis, *J. Biol. Chem.* **1994**, *269*, 13057–13060.
3. B. W. Dijkstra, K. H. Kalk, J. Drenth, G. H. de Haas, M. R. Egmond, A. J. Slotboom, *Biochemistry* **1984**, *23*, 2759–2766.
4. Y. Shi, P. Burn, *Nat. Rev. Drug Discov.* **2004**, *3*, 695–710.
5. R. Homan, K. L. Hamelehle, *J. Lipid Res.* **1998**, *39*, 1197–1209.
6. K. Mackay, J. R. Starr, R. M. Lawn, J. L. Ellsworth, *J. Biol. Chem.* **1997**, *272*, 13380–13389.
7. D. Hollander, D. Morgan, *Lipids* **1980**, *15*, 395–400.
8. S. C. Young, D. Y. Hui, *Biochem. J.* **1999**, *339*, 615–620.
9. K. W. Huggins, A. C. Boileau, D. Y. Hui, *Am. J. Physiol. Endocrinol. Metab.* **2002**, *283*, E994–E1001.
10. S. Marcussi, C. D. Sant'Ana, C. Z. Oliveira, A. Q. Rueda, D. L. Menaldo, R. O. Beleboni, R. G. Stabeli, J. R. Giglio, M.

R. Fontes, A. M. Soares, *Curr. Top. Med. Chem.* **2007**, *7*, 743–756.
11. R. Hyde-DeRuyscher, L. A. Paige, D. J. Christensen, N. Hyde-DeRuyscher, A. Lim, Z.L. Fredericks, J. Kranz, P. Gallant, J. Zhang, S. M. Rocklage, D. M. Fowlkes, P. A. Wendler, P. T. Hamilton, *Chem. Biol.* **2000**, *7*, 17–25.
12. M. Lunder, T. Bratkovič, S. Kreft, B. Štrukelj, *J. Lipid Res.* **2005**, *46*, 1512–1516.
13. M. S. Meiring, D. Litthauer, J. Hársfalvi, V. van Wyk, P. N. Badenhorst, H.F. Kotzé, *Thromb. Res.* **2002**, *107*, 365–371.
14. H. Grøn, R. Hyde-DeRuyscher, *Curr. Opin. Drug Discov. Develop.* **2000**, *3*, 636–645.
15. B. K. Kay, A. V. Kurakin, R. Hyde-DeRuyscher, *Drug Discov. Today* **1998**, *3*, 370–378.
16. M. Lunder, T. Bratkovič, B. Doljak, S. Kreft, U. Urleb, B. Štrukelj, N. Plazar, *Appl. Biochem. Biotechnol.* **2005**, *127*, 125–131.
17. F. Gubenšek, T.R. Pattabhiraman, F.E. Russell, *Toxicon* **1980**, *18*, 699–701.
18. J. Pungerčar, I. Križaj, N.-S. Liang, F. Gubenšek, *Biochem. J.* **1999**, *341*, 139–145.
19. F. Radvanyi, L. Jordan, F. Russo-Marie, C. Bon, *Anal. Biochem.* **1989**, *177*, 103–109.
20. C. C. Leslie, M.H. Gelb, *Methods Mol. Biol.* **2004**, *284*, 229–242.
21. H. B. Lowman, *Annu. Rev. Biophys. Biomol. Struct.* **1997,** *26*, 401–424.
22. N. Singh, R. K. Somvanshi, S. Sharma, S. Dey, P. Kaur, T. P. Singh, *Curr. Top. Med. Chem.* **2007,** *7*, 757–764.
23. W. B. Church, A. S. Inglis, A. Tseng, R. Duell, P. W. Lei, K. J. Bryant, K. F. Scott, *J. Biol. Chem.* **2001**, *276*, 33156–33164.
24. V. Chandra, J. Jasti, P. Kaur, S. Dey, A. Srinivasan, C.H. Betzel, T.P. Singh, *Acta Crystallogr. D Biol. Crystallogr.* **2002**, *58*, 1813–1819.
25. R. K. Singh, P. Vikram, J. Makker, T. Jabeen, S. Sharma, S. Dey, P. Kaur, A. Srinivasan, T. P. Singh, *Biochemistry* **2003,** *42*, 11701–11706.

Povzetek

Pri iskanju ligandov s potencialno inhibitorno aktivnostjo na pankresno fosfolipazo A2 ali amoditoksin C (nevrotoksin iz strupnih žlez modrasa *Vipera ammodytes ammodytes*) smo uporabili dve knjižnici naključnih peptidov, izraženih na bakteriofagih. Peptidi, ki smo jih selekcionirali z različnimi selekcijskimi protokoli, jasno kažejo afiniteto do pankreasne fosfolipaze in amoditoksina C, ki smo jo določili s pomočjo površinske plazmonske resonance in encimskoimunskega testa. Peptidi presenetljivo izkazujejo podobno afiniteto do obeh proteinov, ne glede, katerega smo uporabili kot ciljno molekulo pri selekciji. Navkljub nedvoumni afiniteti, peptidi v območju testiranih koncentracij niso inhibirali fosfolipaznega delovanja *in vitro*.