Linda Christian Carrijo Carvalho

LOPAP (*Lonomia obliqua* prothrombin activator protease): clonagem e expressão em levedura *Pichia pastoris,* obtenção de um peptídeo sintético, análise estrutural e avaliação de suas potenciais aplicações

Tese apresentada ao Programa de Pós-Graduação Interunidades em Biotecnologia USP/Instituto Butantan/IPT, para obtenção do Título de Doutor em Biotecnologia.

Área de concentração:
Bioquímica e Biologia Molecular

Orientadora:
Dra. Ana Marisa Chudzinski-Tavassi

São Paulo

2009

RESUMO

Carrijo Carvalho LC. LOPAP (*Lonomia obliqua* prothrombin activator protease): clonagem e expressão em levedura *Pichia pastoris*, obtenção de um peptídeo sintético, análise estrutural e avaliação de suas potenciais aplicações [Tese]. São Paulo: Programa de Pós-Graduação Interunidades em Biotecnologia da Universidade de São Paulo/Instituto Butantan/ Instituto de Pesquisas Tecnológicas; 2009.

O contato acidental com lagartas da mariposa *Lonomia obliqua* causa envenenamento caracterizado como síndrome hemorrágica devido a uma coagulopatia de consumo. O Lopap é uma das toxinas mais abundantes nas cerdas desta lagarta, caracterizado como uma serino protease que apresenta atividade ativadora de protrombina e atividade antiapoptótica. Esta proteína é o único membro descrito da família das lipocalinas com atividade proteolítica. Membros desta família são conhecidos como carreadores de moléculas lipofílicas, reguladores de processos metabólicos e homeostáticos, além do envolvimento na modulação de respostas celulares, tendo em comum a presença de três domínios conservados na estrutura primária. Tendo em vista potenciais aplicações terapêuticas e biotecnológicas do Lopap, este trabalho teve como objetivo a obtenção do Lopap recombinante (rLopap) por metodologia escalonável e a avaliação de sua atividade *in vivo* e *in vitro*. O Lopap foi clonado a partir do transcriptoma das cerdas de *L. obliqua* e expresso na levedura *Pichia pastoris,* sendo recuperado na forma enzimaticamente ativa e com capacidade procoagulante sobre plasma humano. Em modelo animal de controle sistêmico da hemostasia, o rLopap foi capaz de diminuir o tempo de sangramento em animais anticoagulados com enoxaparina. A partir de domínios conservados de lipocalinas encontrados na seqüência primária do Lopap, peptídeos sintéticos foram obtidos e testados em cultura de células endoteliais. Um peptídeo relacionado ao segundo domínio (*motif 2),* designado *antiapoptotic peptide* (AP), foi capaz de reproduzir os efeitos do Lopap na modulação da sobrevivência celular. Em cultura de

fibroblastos, AP foi capaz de induzir a síntese de proteínas de matriz extracelular como colágeno, fibronectina e tenascina. O aumento de colágeno foi observado também na derme de animais tratados localmente com AP, cujo efeito persistiu por mais de três meses. A ocorrência, em outras proteínas, de seqüências relacionadas a AP foi investigada através da busca em bancos de dados públicos por seqüência de proteínas (blastp) e por domínios conservados (*motif search library*, *blast conserved domain*). Os resultados obtidos mostraram a presença de seqüências similares em lipocalinas de origem humana e animal como purpurina, prostaglandina D sintase e apolipoproteína D e também em proteínas de vírus, bactérias, parasitas e insetos, muitas das quais não tem função descrita. O alinhamento do modelo tridimensional do Lopap com outras lipocalinas descritas com atividade antiapoptótica (purpurina e prostaglandina D sintase) mostrou que a localização e conformação tridimensional da região correspondente a AP têm padrão semelhante às seqüências da purpurina e prostaglandina D sintase. Os valores relativos de polaridade, hidropaticidade e exposição ao solvente dos aminoácidos desta região também foram semelhantes nestas proteínas. Através destes resultados, pode-se sugerir que a seqüência identificada confere uma propriedade comum e conservada em muitas lipocalinas. A desvinculação da ação procoagulante do Lopap através da obtenção de um peptídeo sintético abre perspectivas para seu uso em várias aplicações que se baseiam em sua ação na modulação celular, como um componente cosmético, no reparo e remodelamento tecidual e em várias disfunções que envolvem morte celular e perda de colágeno.

Palavras-chave: Bioquímica. Proteínas recombinantes. Peptídeos. Hemostasia. Biologia celular. Matriz extracelular.

ABSTRACT

Carrijo Carvalho LC. LOPAP (*Lonomia obliqua* prothrombin activator protease): cloning and expression in *Pichia pastoris* yeast, design of a synthetic peptide, structural analysis and evaluation of its potential applications [Doctoral thesis]. São Paulo: Programa de Pós-Graduação Interunidades em Biotecnologia da Universidade de São Paulo/Instituto Butantan/Instituto de Pesquisas Tecnológicas; 2009.

Accidental contact with the *Lonomia obliqua* moth caterpillar causes envenoming outcomes characterized as hemorrhagic syndrome due to a consumption coagulopathy. Lopap is one of the most abundant toxins in the caterpillar's bristles. This protein was characterized as a serine protease which displays prothrombin activation and antiapoptotic activities. Lopap is the only member of the lipocalin family with proteolytic activity. Members of lipocalins family are recognized as carriers of small lipophilic molecules, regulators of methabolic and homeostatic process, by their involvement in the modulation of cells responses, and share three characteristic conserved domains in their primary structure. In view of the potential therapeutic of biotechnological applications of Lopap, this work aimed to obtain recombinant Lopap (rLopap) by a scaled-up methodology and the evaluation of the rLopap's activity *in vivo* and *in vitro*. Lopap was cloned from the transcriptome of the *L. obliqua* bristles and expressed in the *Pichia pastoris* yeast. The recombinant protein was recovered in its enzymatic active form and displayed procoagulante effect on human plasma. rLopap was able to reduce the bleeding time in animals that was anticoagulated with enoxaparin in an experimental model to evaluate systemic hemostasis. Based on lipocalin conserved domains found in Lopap primary sequence, synthetic peptides were obtained and assayed on endothelial cell culture. A peptide related to the second domain (*motif 2),* called antiapoptotic peptide (AP), was able to reproduce the effects of Lopap in the modulation of cell survival. In fibroblast culture, AP was able to induce the synthesis of extracellular matrix proteins, such as collagen,

fibronectin and tenascin. The increase of collagen content was also observed in the dermis of animals locally treated with AP. This effect was observed up to three months after treatment. The presence of AP-related sequences in other proteins was investigated by search at public data banks considering complete protein sequences (blastp) and conserved domains (motif search library, blast conserved domain). Results showed the occurrence of similar sequences among human and animal lipocalins, such as purpurin, prostaglandin D synthase and apolipoprotein D, and also in proteins from virus, bacteria, parasites and insects, many of these have no functions described. Alignment of the Lopap tridimensional model with other antiapoptotic lipocalins (purpurin and prostaglandin D synthase) revealed that the region corresponding to AP sequence have similar tridimensional structures among these proteins. The relative polarity, hydropathicity and solvent accessibility values observed for each amino acid residue of AP and related sequences in the tertiary structure of their proteins also had a similar pattern. These results suggest that AP-related sequence signatures confer a common property in many lipocalins. The trim of the Lopap procoagulant activity by means of obtaining a synthetic peptide open perspectives for its use in several applications that are based merely on its action via cell modulation, for example, as a cosmetic component, aiding tissue repair and remodeling, and in many dysfunctions involving cell death and loss of collagen.

Keywords: Biochemistry. Recombinant proteins. Peptides. Hemostasis. Cell biology. Extracellular matrix.

1 INTRODUÇÃO

Lonomia obliqua é uma espécie de mariposa comumente encontrada na região Sul do Brasil. Em sua fase larval são conhecidas como lagartas ou taturanas e apresentam o corpo coberto por cerdas, que ao contato com a pele secretam veneno com propriedades procoagulantes. O envenenamento causa uma síndrome hemorrágica devido à coagulação intravascular disseminada levando a coagulopatia de consumo (Zannin et al., 2003). O Lopap é uma das toxinas mais abundantes presente nas cerdas destas lagartas (Veiga et al., 2005; Ricci-Silva et al., 2008). Estudos com a proteína nativa demonstraram experimentalmente que o Lopap induz efeitos semelhantes aos observados com o extrato bruto das cerdas da lagarta em animais de laboratório e aos observados no envenenamento humano, causando depleção de fibrinogênio e incoagulabilidade sanguínea (Reis et al., 1999, 2001a). Neste sentido, o Lopap tem sido apontado como uma das principais toxinas presentes no veneno de L. obliqua (Carrijo-Carvalho e Chudzinski-Tavassi, 2007).

O Lopap foi inicialmente caracterizado como um ativador de protrombina com atividade do tipo serino protease (Reis et al., 2001b). Contudo, esta proteína não apresenta similaridade com nenhum ativador de protrombina ou serino proteases conhecidos, mas tem domínios conservados na seqüência primária característicos de lipocalinas e sua estrutura secundária e terciária segue padrão semelhante a membros desta família, com a predominância de folhas β-pregueadas formando uma estrutura molecular em forma de cálice ou barril (Reis et al., 2006). O envolvimento de algumas lipocalinas em mecanismos de reparo, remodelamento tecidual (Olsson, 1997; Rassart et al., 2000; Descalzi Cancedda et al., 2000; Sousa et al., 2005; Hemdahl et al., 2006) e sobrevivência celular (Schubert et al., 1986; Taniike et al., 2002; Tong et al., 2005) tem sido proposto. Em estudos prévios com o Lopap nativo em cultura de células endoteliais que inicialmente foram realizados para avaliar possíveis efeitos sobre a modulação da hemostasia independente

de fatores da coagulação, foi observado que o Lopap apresentava também uma atividade citoprotetora (Fritzen et al., 2005). Particularmente, a indução da sobrevivência celular não é uma propriedade esperada para uma toxina. Desta forma, todas estas características indicam que o Lopap representa uma molécula com características únicas, que despertam interesse tanto científico - relativo às particularidades bioquímicas e estruturais desta proteína, quanto terapêutico e biotecnológico, relacionado à caracterização das propriedades desta molécula e potenciais aplicações. No início deste trabalho, haviam três patentes depositadas no Instituto Nacional da Propriedade Industrial (INPI) com fase internacional (PCT) em co-titularidade com a FAPESP, com base nas diferenças estruturais que permitem enquadrar o Lopap como um novo ativador de protrombina, sua atividade procoagulante, em seu potencial desfibrinogenante, e em sua atividade citoprotetora (Chudzinski-Tavassi e Reis, 2002; Chudzinski-Tavassi et al., 2004, 2005).

Um dos fatores limitantes ao estudo do Lopap é sua obtenção. A obtenção do Lopap nativo é limitada a pequenas quantidades purificadas a partir do extrato das cerdas das lagartas de *L. obliqua*; à disponibilidade sazonal destas lagartas, restrita aos meses mais quentes do ano; e à coleta e transporte das mesmas, a partir de cidades da região Sul do Brasil. A obtenção do Lopap na forma recombinante é limitada pela possibilidade de manutenção das atividades enzimática e celular na proteína recuperada ao final dos processos de expressão e purificação, e pela necessidade de uma metodologia escalonável.

A partir de uma biblioteca de genes transcritos nas cerdas de *L. obliqua* (Reis, 2002), o Lopap foi clonado e obtido na forma recombinante (rLopap) monomérica pela expressão em cepa de *Escherichia coli* com cauda de poli-histidina, na forma de corpúsculos de inclusão (Reis et al., 2006). As limitações para obtenção do rLopap pelo sistema bacteriano descrito são: a obtenção da proteína com um enovelamento correto, pois a produção em corpúsculos de inclusão implica em uma fase de desnaturação seguida de renaturação *in vitro*; a baixa atividade catalítica

da proteína obtida; a ausência de processamentos pós-traducionais; a presença de uma cauda de poli-histidina na proteína recombinante recuperada. Todos estes aspectos mostram a importância do desenvolvimento e aprimoramento de metodologias de obtenção do rLopap com especial atenção quanto à estabilidade da molécula.

Este trabalho teve como objetivo geral o desenvolvimento do Lopap e o estudo de suas potenciais aplicações. Desta forma, nossos estudos foram dedicados à obtenção do Lopap recombinante em sistema de expressão na levedura *Pichia pastoris*, através de processo escalonável, em avaliar os efeitos desta proteína *in vitro* e *in vivo* e, por outro lado, através do mapeamento peptídico do Lopap, em identificar a região da molécula envolvida nos efeitos do Lopap ao nível celular, e caracterizá-la com relação à atividade catalítica do Lopap e com relação aos domínios conservados em outros membros da família das lipocalinas. Os objetivos específicos foram os seguintes:

1) expressar o Lopap na forma solúvel em *E. coli* Origami;

2) clonar e expressar o Lopap na levedura *Pichia pastoris;*

3) estabelecer um protocolo padrão de produção do rLopap na levedura *P. pastoris*, por um processo escalonável, com produção em alta densidade celular em biorreator;

4) estudar a estabilidade do rLopap;

5) avaliar a atividade procoagulante e antiapoptótica do rLopap;

6) avaliar experimentalmente a ação do rLopap como agente hemostático em animais;

7) entender como os domínios moleculares do Lopap estão envolvidos na sua ação celular - através da avaliação da atividade de peptídeos sintéticos derivados de domínios conservados da proteína;

8) avaliar a ação de peptídeos sintéticos derivados do Lopap sobre a síntese de moléculas de matriz extracelular em cultura de células e na derme de animais.

6 CONCLUSÕES

- A metodologia utilizada permitiu a obtenção do rLopap na levedura *P. pastoris,* com alta atividade específica, por um processo escalonável e reprodutível;

- O tratamento com o rLopap foi efetivo na diminuição do tempo de sangramento em coelhos anticoagulados com enoxaparina;

- A atividade procoagulante do Lopap foi desvinculada da atividade antiapoptótica através da obtenção de peptídeos sintéticos derivados da seqüência primária do Lopap;

- Uma seqüência peptídica de sete aminoácidos foi identificada como a região envolvida na ação do Lopap em células, promovendo a sobrevivência celular e induzindo a síntese de proteínas de matriz extracelular;

- A seqüência peptídica identificada está presente em outras lipocalinas e proteínas hipotéticas, as quais podem compartilhar propriedades biológicas comuns.

REFERÊNCIAS*

Ahmad S, Gromiha M, Fawareh H, Sarai A. ASAView: database and tool for solvent accessibility representation in proteins. BMC Bioinformatics. 2004;5:51.

Al-Daghri NM, Al-Attas OS, Alokail M, Draz HM, Bamakhramah A, Sabico S. Retinol binding protein-4 is associated with TNF-alpha and not insulin resistance in subjects with type 2 diabetes mellitus and coronary heart disease. Dis Markers. 2009;26:135-40.

Aldo Filho, D. Avaliação da expressão dos receptores de interleucina-8, CXCR1 e CXCR2, e da atividade proliferativa em fibroblastos de quelóide e de pele normal [tese]. São Paulo: Faculdade de Medicina da Universidade de São Paulo;2006.

Ali YB, Carrière F, Abousalham A. High-level constitutive expression in *Pichia pastoris* and one-step purification of phospholipase D from cowpea (*Vigna unguiculata* L. Walp). Protein Expr Purif. 2007;51:162-9.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403-10.

Alvarez Flores MP, Fritzen M, Reis CV, Chudzinski-Tavassi AM. Losac, a factor X activator from *Lonomia obliqua* bristle extract: its role in the pathophysiological mechanisms and cell survival. Biochem Biophys Res Commun. 2006;343:1216-23.

Arocha-Piñango CL, Layrisse M. Fibrinolysis produced by contact with caterpillar. Lancet. 1969;19:810-2.

Arocha-Piñango CL, Pepper DS. Studies of a fibrinolytic enzyme from the larvae of *Lonomia achelous* (Cramer) using chromogenic substrates. Thromb Haemost. 1981;46:710-3.

*De acordo com: International Commitee of Medical Journal Editors. Uniform Requirements for Manuscripts Submitted to Biomedical Journal: Sample References. Available from: http://www.icmje.org [2007 May 22].

Arocha-Piñango CL, De Bosch NB, Torres A, Goldstein C, Nouel AL, Arguello A, et al. Six new cases of caterpillar-induced bleeding syndrome. Thromb Haemost. 1992;67:402-7.

Arocha-Piñango CL, Marval E, Guerrero B. *Lonomia* genus caterpillar toxins: biochemical aspects. Biochimie. 2000;82:937-42.

Bedard PA, Yannoni Y, Simmons DL, Erikson RL. Rapid repression of quiescence-specific gene expression by epidermal growth factor, insulin, and pp60v-src. Mol Cell Biol. 1989;9(3):1371-5.

Berman P, Gray P, Chen E, Keyser K, Ehrlich D, Karten H, et al. Sequence analysis, cellular localization, and expression of a neuroretina adhesion and cell survival molecule. Cell. 1987;51(1):135-42.

Bernstein EF, Uitto J. The Effect of photodamage on dermal extracellular matrix. Clin Dermatol. 1996;14:143-51.

Bishop RE. The bacterial lipocalins. Biochim Biophys Acta. 2000;1482:73-83.

Bishop RE, Penfold SS, Frost LS, Höltje JV, Weiner JH. Stationary phase expression of a novel *Escherichia coli* outer membrane lipoprotein and its relationship with mammalian apolipoprotein D. J Biol Chem. 1995;270: 23097–103.

Bohrer CB, Reck Junior J, Fernandes D, Sordi R, Guimarães JA, Assreuy J, Termignoni C. Kallikrein–kinin system activation by *Lonomia obliqua* caterpillar bristles: involvement in edema and hypotension responses to envenomation. Toxicon. 2006;49:663-9.

Bradford M. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem. 1976;72:248-54.

Brucato CL, Birr CA, Bruguera P, Ruiz JA, Sánchez-Martínez D. Expression of recombinant rabbit tissue factor in Pichia pastoris, and its application in a prothrombin time reagent. Protein Expr Purif. 2002;26(3):386-93.

Bugos RC, Hieber AD, Yamamoto HY. Xanthophyll cycle enzymes are members of the lipocalin family, the first identified from plants. J Biol Chem. 1998;273:15321-4.

Byun Y, Chang LC, Lee LM, Han IS, Singh VK, Yang VC. Low molecular weight protamine: a potent but nontoxic antagonist to heparin/low molecular weight protamine. ASAIO J. 2000;46(4):435-9.

Cancedda FD, Dozin B, Rossi F, Molina F, Cancedda R, Negri A, Ronchi S. The Ch21 protein, developmentally regulated in chick embryo, belongs to the superfamily of lipophilic molecule carrier proteins. J Biol Chem. 1990; 265(31):19060-4.

Carrijo-Carvalho LC, Chudzinski-Tavassi AM. The venom of the *Lonomia* caterpillar: an overview. Toxicon. 2007;49:741-57.

Carter NJ, McCormack PL, Plosker GL. Enoxaparin: a review of its use in ST-segment elevation myocardial infarction. Drugs. 2008;68(5):691-710.

Caovilla JJ, Barros EJ. Efficacy of two different doses of antilonomic serum in the resolution of hemorrhagic syndrome resulting from envenoming by *Lonomia obliqua* caterpillars: a randomized controlled trial. Toxicon. 2004; 43(7):811-8.

Castro Bastos L, Veiga ABG, Guimarães JA, Tonussi CR. Nociceptive and edematogenic responses elicited by a crude bristle extract of *Lonomia obliqua* caterpillars. Toxicon. 2004;43:273-8.

Cereghino JL, Cregg JM. Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*. FEMS Microbiol Rev. 2000;24(1):45-66.

Chan S, Kong M, Minning DM, Hedner U, Marder VJ. Assessment of recombinant factor VIIa as an antidote for bleeding induced in the rabbit by low molecular weight heparin. J Thromb Haemost. 2003;1(4):760-5.

Charron JB, Breton G, Badawi M, Sarhan F. Molecular and structural analyses of a novel temperature stressinduced lipocalin from wheat and Arabidopsis. FEBS Lett. 2002;517:129-32.

Chen CC, Wu PH, Huang CT, Cheng KJ. A *Pichia pastoris* fermentation strategy for enhancing the heterologous expression of an *Escherichia coli* phytase. Enzyme Microb Technol. 2004;35:315-20.

Chen YH, Chang TC, Chang GG. Functional expression, purification, and characterization of the extra stable human placental alkaline phosphatase in the *Pichia pastoris* system. Protein Expr Purif. 2004;36:90-9.

Choi CW, Nam KP, Seo DH, Choi JW, Lee CS, Kim HR, Yun CY. Comparative analysis of two biliproteins, BP1 and BP2, from haemolymph of cabbage white butterfly, Pieris rapae. Arch Insect Biochem Physiol. 2006;61(4):220-30.

Chudzinski-Tavassi AM, Schattner M, Fritzen M, Pozner RG, Reis CV, Lourenço D, Lazzari MA. Effects of Lopap on human endothelial cells and platelets. Haemostasis. 2001;31:257-65.

Chudzinski-Tavassi AM, Reis CV, inventors. Processo de purificação de proteínas solúveis das cerdas da lagarta *L. obliqua* com atividade ativadora de protrombina; processo para determinação parcial da seqüência de aminoácidos do ativador de protrombina; processo de determinação da atividade ativadora de protrombina da fração II, seqüência N-terminal e seqüência de fragmentos internos da fração ativadora de protrombina, ativador de protrombina e uso do ativador de protrombina. BR patent PI02002698. 2002.

Chudzinski-Tavassi AM, Reis CV, Ho PL, Ramos CR, Farsky S, Fritzen M, inventors. Processo de obtenção de protease ativadora de protrombina recombinante (Lopap) na forma monomérica; protease ativadora de protrombina recombinante (Lopap), seqüência de aminoácidos da proteína recombinante assim obtida e uso como agente desfibrinogenante e kit diagnóstico para desprotrombinemias. BR patent PI04038827. 2004.

Chudzinski-Tavassi AM, Alvarez Flores MP. Exploring new molecules and activities from *Lonomia obliqua* caterpillars. Pathophysiol Haemost Thromb. 2005;34:228-33.

Chudzinski-Tavassi AM, Falci M, Maria DA, Reis CV, inventors. Composições farmacêuticas baseadas em Lopap e usos das ditas composições. BR patent PI0504199-6. 2005.

Chudzinski-Tavassi AM, Carrijo-Carvalho LC. Biochemical and biological properties of *Lonomia obliqua* bristle extract. J Venom Anim Toxins Incl Trop Dis. 2006;12(2):156-171.

Cregg JM, Barringer KJ, Hessler AY, Madden KR. Pichia pastoris as a host system for transformations. Mol Cell Biol. 1985 Dec;5(12):3376-85.

Damasceno LM, Anderson KA, Ritter G, Cregg JM, Old LJ, Batt CA. Cooverexpression of chaperones for enhanced secretion of a single-chain antibody fragment in *Pichia pastoris*. Appl Microbiol Biotechnol. 2007;74(2):381-9.

DeLano WL. The PyMOL Molecular Graphics System (2002). [página na Internet]. Palo Alto, CA, USA: DeLano Scientific. Disponível em: http://www.pymol.org [2009 Ago 16].

Descalzi Cancedda F, Manduca P, Tacchetti C, Fossa P, Quarto R, Cancedda R. Developmentally regulated synthesis of a low molecular weight protein (Ch 21) by differentiating chondrocytes. J Cell Biol. 1988; 107(6 Pt 1):2455-63.

Descalzi Cancedda F, Dozin B, Zerega B, Cermelli S, Cancedda R. Ex-FABP: a fatty acid binding lipocalin developmentally regulated in chicken endochondral bone formation and myogenesis. Biochim Biophys Acta. 2000;1482(1-2):127-35.

Descalzi Cancedda F, Dozin B, Zerega B, Cermelli S, Gentili C, Cancedda R. Ex-FABP, extracellular fatty acid binding protein, is a stress lipocalin expressed during chicken embryo development. Mol Cell Biochem. 2002; 239(1-2):221-5.

Dias-da-Silva W, Rocha-Campos ACM, Gonçalves LRC, Souza-e-Silva MC, Higashi HG, Yamagushi IK, Kelen EMA. Development of an antivenom against toxins of *Lonomia obliqua* caterpillars. Toxicon. 1996;34:1045-9.

Diaz JH. The evolving global epidemiology, syndrome classification, management, and prevention of caterpillar envenoming. Am J Trop Med Hyg. 2005;72:347-57.

Do Carmo S, Jacomy H, Talbot PJ, Rassart E. Neuroprotective effect of apolipoprotein D against human coronavirus OC43-induced encephalitis in mice. J Neurosci. 2008;28(41):10330-8.

Donato JL, Moreno RA, Hyslop S, Duarte A, Antunes E, Le Bonniec BF, et al.. *Lonomia obliqua* caterpillar trigger human blood coagulation via activation of factor X and prothrombin. Thromb Haemost. 1998;79:539-42.

Dozin B, Descalzi F, Briata L, Hayashi M, Gentili C, Hayashi K, et al. Expression, regulation, and tissue distribution of the Ch21 protein during chicken embryogenesis. J Biol Chem. 1992;267(5):2979-85.

Du G, Prestwich GD. Protein structure encodes the ligand binding specificity in pheromone binding proteins. Biochemistry. 1995; 34(27):8726-32.

Duarte CA, Caovilla J, Lorini I, Lorini D, Mantorani G, Sumida J, et al.. Insuficiencia renal aguda por acidentes com lagartas. J Bras Nefrol. 1990; 12:184-7.

Eichinger A, Nasreen A, Kim HJ, Skerra A. Structural insight into the dual ligand specificity and mode of high density lipoprotein association of apolipoprotein D. J Biol Chem. 2007;282(42):31068-75.

Filippovich I, Sorokina N, St Pierre L, Flight S, de Jersey J, Perry N, et al. Cloning and functional expression of venom prothrombin activator protease from *Pseudonaja textilis* with whole blood procoagulant activity. Br J Haematol. 2005;131(2):237-46.

Flower DR, North AC, Attwood TK. Structure and sequence relationships in the lipocalins and related proteins. Protein Sci. 1993;2(5):753-61.
Flower DR. The lipocalin protein family: a role in cell regulation. FEBS Lett. 1994;354(1):7-11.

Flower DR. The lipocalin protein family: structure and function. Biochem J. 1996;318(Pt 1):1-14.

Fox JW, Serrano SMT. Structural considerations of the snake venom metalloproteinases, key members of the M12 reprolysin family of metalloproteinases. Toxicon. 2005;45:969–85.

Fritzen M. Caracterização funcional do LOPAP (ativador de protrombina da lagarta *Lonomia obliqua*) [dissertação]. São Paulo: Universidade Federal de São Paulo; 2002.

Fritzen M, Flores MPA, Reis CV, Chudzinski-Tavassi AM. A prothrombin activator (Lopap) modulating inflamation, coagulation and cell survival mechanisms. Biochem Biophys Res Commun. 2005;333:517-23.

Gasser B, Sauer M, Maurer M, Stadlmayr G, Mattanovich D. Transcriptomics-based identification of novel factors enhancing heterologous protein secretion in yeasts. Appl Environ Microbiol. 2007;73(20):6499-507.

Geer LY, Domrachev M, Lipman DJ, Bryant SH. CDART: protein homology by domain architecture. Genome Res. 2002;12:1619-23.

Gellissen G. Heterologous protein production in methylotrophic yeasts. Appl Microbiol Biotechnol. 2000;54:741-50.

Giannoni P, Zambotti A, Pagano A, Cancedda R, Dozin B. Differentiation-dependent activation of the extracellular fatty acid binding protein (Ex-FABP) gene during chondrogenesis. J Cell Physiol. 2004;198(1):144-54.

Glasgow BJ, Gasymov OK, Abduragimov AR, Yusifov TN, Altenbach C, Hubbell WL. Side chain mobility and ligand interactions of the G strand of tear lipocalins by site-directed spin labeling. Biochemistry. 1999; 38:13707-16.

Goodman WG, Adams B, Trost JT. Purification and characterization of a biliverdin-associated protein from the hemolymph of *Manduca sexta*. Biochemistry. 1985;24(5):1168-75.

Gouveia AICB, Silveira RB, Nader HB, Dietrich CP, Gremski W, Veiga SS. Identification and partial characterisation of hyaluronidases in *Lonomia obliqua* venom. Toxicon. 2005;45:403-10.

Grantham R. Amino acid difference formula to help explain protein evolution. Science. 1974;185:862-4.

Gross PL, Weitz JI. New anticoagulants for treatment of venous thromboembolism. Arterioscler Thromb Vasc Biol. 2008;28:380-6.

Grzyb J, Latowski D, Strzałka K. Lipocalins - a family portrait. J Plant Physiol. 2006;163(9):895-915.

Gudderra NP, Ribeiro JM, Andersen JF. Structural determinants of factor IX(a) binding in nitrophorin 2, a lipocalin inhibitor of the intrinsic coagulation pathway. J Biol Chem. 2005;280:25022-8.

Guerrero B, Arocha-Piñango CL, Gil San Juan A. Degradation of human factor XIII by lonomin V, a purified fraction of *Lonomia achelous* caterpillar venom. Thromb Res. 1997;87:171-81.

Guo W, González-Candelas L, Kolattukudy PE. Cloning of a novel constitutively expressed pectate lyase gene pelB from *Fusarium solani* f. sp. pisi (*Nectria haematococca*, mating type VI) and characterization of the gene product expressed in *Pichia pastoris*. J Bacteriol. 1995;177(24):7070-7.

Gurramkonda C, Adnan A, Gäbel T, Lünsdorf H, Ross A, Nemani SK et al. Simple high-cell density fed-batch technique for high-level recombinant protein production with *Pichia pastoris*: Application to intracellular production of Hepatitis B surface antigen. Microb Cell Fact. 2009;8:13.

Haas S. New oral Xa and IIa inhibitors: updates on clinical trial results. J Thromb Thrombolysis. 2008;25:52-60.

Hasson SS, Theakston RDG, Harrison RA. Cloning of a prothrombin activator-like metalloproteinase from the West African saw-scaled viper, *Echis ocellatus*. Toxicon. 2003;42:629-34.

Heimo H, Palmu K, Suominen I. Human placental alkaline phosphatase: expression in Pichia pastoris, purification and characterization of the enzyme. Protein Expr Purif. 1998;12(1):85-92.

Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, et al. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol. 2006; 26(1):136-42.

Henze A, Frey SK, Raila J, Tepel M, Scholze A, Pfeiffer AF, et al. Evidence that kidney function but not type 2 diabetes determines retinol-binding protein 4 serum levels. Diabetes. 2008;57:3323-26.

Hieber AD, Bugos RC, Yamamoto HY. Plant lipocalins: violaxanthin de-epoxidase and zeaxanthin epoxidase. Biochim Biophys Acta. 2000; 1482(1-2):84-91.

Holden HM, Law JH, Rayment I. Crystallization of insecticyanin from the hemolymph of the tobacco hornworm *Manduca sexta* L. in a form suitable for a high resolution structure determination. J Biol Chem. 1986; 261(9):4217-8.

Holden HM, Rypniewski WR, Law JH, Rayment I. The molecular structure of insecticyanin from the tobacco hornworm *Manduca sexta* L. at 2.6 A resolution. EMBO J. 1987;6(6):1565-70.

Hunt D. Low molecular weight heparins in clinical practice. Southern Med J. 1998;91:2-10.

Huo X, Liu Y, Wang X, Ouyang P, Niu Z, Shi Y, Qiu B. Co-expression of human protein disulfide isomerase (hPDI) enhances secretion of bovine follicle-stimulating hormone (bFSH) in Pichia pastoris. Protein Expr Purif. 2007;54(2):234-9.

Iannetti A, Pacifico F, Acquaviva R, Lavorgna A, Crescenzi E, Vascotto C et al. The neutrophil gelatinase-associated lipocalin (NGAL), a NF-kappaB-regulated gene, is a survival factor for thyroid neoplastic cells. Proc Natl Acad Sci U S A. 2008;105(37):14058-63.

Inan M, Aryasomayajula D, Sinha J, Meagher MM. Enhancement of protein secretion in Pichia pastoris by overexpression of protein disulfide isomerase. Biotechnol Bioeng. 2006;93(4):771-8.

Irikura D, Kumasaka T, Yamamoto M, Ago H, Miyano M, Kubata KB, et al. Cloning, expression, crystallization, and preliminary X-ray analysis of recombinant mouse lipocalin-type prostaglandin D synthase, a somnogen-producing enzyme. J Biochem. 2003;133(1):29-32.

Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest. 1973;52:2745-56.

Jurzak M, Latocha M, Gojniczek K, Kapral M, Garncarczyk A, Pierzchała E. Influence of retinoids on skin fibroblasts metabolism in vitro. Acta Pol Pharm. 2008;65(1):85-91.

Kamei H, Ohira T, Yoshiura Y, Uchida N, Nagasawa H, Aida K. Expression of a biologically active recombinant follicle stimulating hormone of Japanese Eel *Anguilla japonica* using methylotropic yeast, *Pichia pastoris*. Gen Comp Endocrinol. 2003;134(3):244-54.

Kang Y, Kulakosky PC, van Antwerpen R, Law JH. Sequestration of insecticyanin, a blue hemolymph protein, into the egg of the hawkmoth *Manduca sexta*. Evidence for receptor-mediated endocytosis. Insect Biochem Mol Biol. 1995;25(4):503-10.

Kang Y, Ziegler R, van Antwerpen R, Law JH. Characterization of the solubilized oocyte membrane receptor for insecticyanin, a biliprotein of the hawkmoth, *Manduca sexta*. Biochim Biophys Acta. 1997;1324(2):285-95.

Kelen EMA, Picarelli ZP, Duarte AC. Hemorrhagic syndrome induced by contact with caterpillars of the genus *Lonomia* (Saturniidae, Hemileucinae). J Toxicol Toxin Rev. 1995;14:283-308.

Kim HJ, Je HJ, Cheon HM, Kong SY, Han J, Yun CY, et al. Accumulation of 23 kDa lipocalin during brain development and injury in *Hyphantria cunea*. Insect Biochem Mol Biol. 2005;35:1133-41.

Kini RM, Rao VS, Joseph JS. Procoagulant proteins from snake venoms. Haemostasis. 2001;31:218-24.

Kini RM. The intriguing world of prothrombin activators from snake venom. Toxicon. 2005;45:1133-45.

Kobayashi K. Summary of recombinant human serum albumin development. Biologicals. 2006;34(1):55-9.

Kornalik F, Blomback B. Prothrombin activation induced by Ecarin - a prothrombin converting enzyme from *Echis carinatus* venom. Thromb Res. 1975;6(1):57–63.

Kyoto University Bioinformatics Center. GenomeNet bioinformatic tools – MOTIF sequence motif search [página na Internet]. Kyoto (Japão): Kyoto University. Disponível em: http://motif.genome.jp/ [2009 Mar 2].

Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol. 1982;157:105-32.

Lattard V, Fondeur-Gelinotte M, Gulberti S, Jacquinet JC, Boudrant J, Netter P, et al. Purification and characterization of a soluble form of the recombinant human galactose-β1,3-glucuronosyltransferase I expressed in the yeast *Pichia pastoris.* Protein Expr Purif. 2006;47:137-43.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-5.

Lemaire C. Descriptions d'Attacidae nouveaux d'Amérique Centrale et du Sud. (Lep.). I. Descriptions preliminaires de 16 espécies nouvelles du genre *Lonomia* Walker. Bull Soc Entomol France. 1972a;76:197-205.

Lemaire C. Revision du genre *Lonomia* Walker (Lep. Attacidae). Ann Soc Entomol France. 1972b;8:767-861.

Li W, Riddiford LM. Two distinct genes encode two major isoelectric forms of insecticyanin in the tobacco hornworm, *Manduca sexta*. Eur J Biochem. 1992;205(2):491-9.

Li WC, Riddiford LM. The two duplicated insecticyanin genes, ins-a and ins-b are differentially expressed in the tobacco hornworm, *Manduca sexta*. Nucleic Acids Res. 1994;22(15):2945-50.

Liao YF, Lal A, Moremen KW. Cloning, expression, purification, and characterization of the human broad specificity lysosomal acid alpha-mannosidase. J Biol Chem. 1996;271(45):28348-58.

Liot G, Roussel BD, Lebeurrier N, Benchenane K, López-Atalaya JP, Vivien D, Ali C. Tissue-type plasminogen activator rescues neurones from serum deprivation-induced apoptosis through a mechanism independent of its proteolytic activity. J Neurochem. 2006;98:1458-64.

Liu R, Lin Q, Sun Y, Lu X, Qiu Y, Li Y, Guo X. Expression, purification, and characterization of hepatitis B virus surface antigens (HBsAg) in yeast Pichia Pastoris. Appl Biochem Biotechnol. 2009;158(2):432-44.

Lögdberg L, Wester L. Immunocalins: a lipocalin subfamily that modulates immune and inflammatory responses. Biochim Biophys Acta. 2000; 1482(1-2):284-97.

Lorini LM, Corseul E. Aspectos Morfológicos de *Lonomia obliqua* Walker (Lepidoptera: Saturniidae). Neotrop Entomol. 2001;30:373-8.

Lucena S, Guerrero B, Salazar AM, Gil A, Arocha-Piñango CL. Degradation of extracellular matrix proteins (fibronectin, vitronectin and laminin) by serine-proteinases isolated from Lonomia achelous caterpillar hemolymph. Blood Coagul Fibrinolysis. 2006;17(6):427-35.

Lucena S, Salazar AM, Gil A, Arocha-Piñango CL, Guerrero B. The action of Lonomin V (*Lonomia achelous*) on fibronectin functional properties. Thromb Res. 2008;121(5):653-61.

Macauley-Patrick S, Fazenda ML, McNeil B, Harvey LM. Heterologous protein production using the *Pichia pastoris* expression system. Yeast. 2005;22:249-70.

Maesaka JK, Palaia T, Frese L, Fishbane S, Ragolia L. Prostaglandin D(2) synthase induces apoptosis in pig kidney LLC-PK1 cells. Kidney Int. 2001;60(5):1692-8.

Mao PL, Beauchemin M, Bédard PA. Quiescence-dependent activation of the p20K promoter in growth-arrested chicken embryo fibroblasts. J Biol Chem. 1993;268(11):8131-9.

Marsh NA. Diagnostic uses of snake venom. Haemostasis. 2001;31(3-6):211-7.

Mauchamp B, Royer C, Garel A, Jalabert A, Da Rocha M, Grenier AM, et al. Polycalin (chlorophyllid A binding protein): a novel, very large fluorescent lipocalin from the midgut of the domestic silkworm *Bombyx mori* L. Insect Biochem Mol Biol. 2006;36(8):623-33.

Melhus H, Bavik CO, Rask L, Peterson PA, Eriksson U. Epitope mapping of a monoclonal antibody that blocks the binding of retinol-binding protein to its receptor. Biochem Biophys Res Commun. 1995;210:105-12.

Modesto JCA, Junqueira-de-Azevedo ILM, Neves-Ferreira AGC, Fritzen M, Oliva MLV, Ho PL, et al. Insularinase A, a prothrombin activator from *Bothrops insularis* venom, is a metalloprotease derived from a gene encoding protease and disintegrin domains. Biol Chem. 2005;386:589-600.

Moon HI, Kim MR, Woo ER, Chung JH. Triterpenoid from *Styrax japonica* SIEB. *et* ZUCC, and its effects on the expression of matrix metalloproteinases-1 and type 1 procollagen caused by ultraviolet irradiated cultured primary human skin fibroblasts. Biol Pharm Bull. 2005; 28(10):2003-6.

Moraes RHP. Identificação dos inimigos naturais de *Lonomia obliqua* Walker, 1855 (Lepidóptera Saturniidae) e possíveis fatores determinantes do aumento da sua população [tese]. São Paulo: Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo; 2002.

Morel N, Massoulié J. Expression and processing of vertebrate acetylcholinesterase in the yeast *Pichia pastoris*. Biochem J. 1997;328(Pt 1):121-9.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. J Immunol Methods. 1983; 65:55-63.

Mozley D, Remberg A, Gärtner W. Large-scale generation of affinity-purified recombinant phytochrome chromopeptide. Photochem Photobiol. 1997;66(5):710-5.

Nagata A, Suzuki Y, Igarashi M, Eguchi N, Toho H, Urade Y, Hayaishi O. Human brain prostaglandin D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins. Proc Natl Acad Sci USA. 1991;88:4020-24.

Navarro A, Ordóñez C, Martínez E, Pérez C, Astudillo A, Tolivia J,. Apolipoprotein D expression absence in degenerating neurons of human central nervous system. Histol Histopathol. 2008;23(8):995-1001.

Nico-Farber K, Harder W, Ab G, Veenhuis M. Methylotrophic yeasts as factories for the production of foreign proteins. Yeast. 1995;11:1331-44.

North AC. Three-dimensional arrangement of conserved amino acid residues in a superfamily of specific ligand-binding proteins. Int J Biol Macromol. 1989;11:56-8.

Nourizad N, Ehn M, Gharizadeh B, Hober S, Nyrén P. Methylotrophic yeast *Pichia pastoris* as a host for production of ATP-diphosphohydrolase (apyrase) from potato tubers (*Solanum tuberosum*). Protein Expr Purif. 2003;27(2):229-37.

Ofosu FA, Freedman J, Semple JW. Plasma-derived biological medicines used to promote haemostasis. Thromb Haemost. 2008;99(5):851-62.

Olsson JE. Human beta-trace in normal and pathological CNS tissues, genital organs and body fluids. Adv Exp Med Biol. 1997;433:351-4.

Pagano A, Giannoni P, Zambotti A, Randazzo N, Zerega B, Cancedda R, Dozin B. CALbeta, a novel lipocalin associated with chondrogenesis and inflammation. Eur J Cell Biol. 2002;81(5):264-72.

Pagano A, Crooijmans R, Groenen M, Randazzo N, Zerega B, Cancedda R, Dozin B. A chondrogenesis-related lipocalin cluster includes a third new gene, CALgamma. Gene. 2003;305(2):185-94.

Peitsch MC, Boguski MS. Is apolipoprotein D a mammalian bilin-binding protein? New Biol. 1990;2(2):197-206.

Pieper U, Eswar N, Webb BM, Eramian D, Kelly L, Barkan DT, et al. MODBASE, a database of annotated comparative protein structure models and associated resources. Nucleic Acids Res. 2009;37(Database issue):D347-54.

Pinto AFM, Silva KRLM, Guimarães JA. Proteases from *Lonomia obliqua* venomous secretions: comparison of procoagulant, fibrin(ogen)olytic and amidolytic activities. Toxicon. 2006;47:113–21.

Pinto AFM, Dragulev B, Guimarães JA, Fox JW. Novel perspectives on the pathogenesis of *Lonomia obliqua* caterpillar envenomation based on assessment of host response by gene expression analysis. Toxicon. 2008; 51(6):1119-28.

Porres JM, Benito MJ, Lei XG. Functional expression of keratinase (kerA) gene from *Bacillus licheniformis* in *Pichia pastoris*. Biotechnol Let. 2002;24:631–6.

Prezoto BC, Maffei FHA, Mattar L, Chudzinski-Tavassi AM, Curi PR. Antithrombotic effect of *Lonomia obliqua* caterpillar bristle extract on experimental venous thrombosis. Braz J Med Biol Res. 2002;35(6):703-12.

Ragolia L, Hall CE, Palaia T. Post-translational modification regulates prostaglandin D2 synthase apoptotic activity: characterization by site-directed mutagenesis. Prostaglandins Other Lipid Mediat. 2007;83(1-2):25-32.

Ramos AT, Gonçalves LRC, Ribeiro OG, Rocha Campos AC, Sant'Anna AO. Effects of *Lonomia obliqua* (lepidoptera, saturniidae) toxin on clotting, inflammatory and antibody responsiveness in genetically selected lines of mice. Toxicon. 2004;43:761-8.

Ramos CR, Abreu PA, Nascimento AL, Ho PL. A high-copy T7 *Escherichia coli* expression vector for the production of recombinant proteins with a minimal N-terminal His-tagged fusion peptide. Braz J Med Biol Res. 2004; 37(8):1103-9.

Rassart E, Bedirian A, Do Carmo S, Guinard O, Sirois J, Terrisse L, Milne R. Apolipoprotein D. Biochim Biophys Acta. 2000;1482(1-2):185-98.

Reis CV, Kelen EMA, Farsky SHP, Portaro FCV, Sampaio CAM, Fernandes BL, et al. A Ca^{++} activated serine protease (Lopap) could be responsible for the haemorragic syndrome caused by the caterpillar *Lonomia obliqua*. Lancet. 1999;353:1942.

Reis CV, Farsky SHP, Fernandes BL, Santoro ML, Oliva MLV, Mariano M, Chudzinski-Tavassi AM. In vivo characterization of Lopap, a prothrombin activator serine protease from the *Lonomia obliqua* caterpillar venom. Thromb Res. 2001a;102:437-43.

Reis CV, Portaro FCV, Andrade SA, Fritzen M, Fernandes B, Sampaio CAM et al. A prothrombin activator serine protease from the *Lonomia obliqua* caterpillar venom (Lopap): biochemical characterization. Thromb Res. 2001b;102:427-36.

Reis CV. Clonagem, sequenciamento, expressão e caracterização parcial da estrutura do Lopap, um ativador de protrombina da lagarta *Lonomia obliqua* [tese]. São Paulo: Universidade Federal de São Paulo; 2002.

Reis CV, Andrade SA, Ramos OH, Ramos CR, Ho PL, Batista IF, Chudzinski-Tavassi AM. Lopap, a prothrombin activator from *Lonomia obliqua* belonging to the lipocalin family: recombinant production, biochemical characterization and structure-function insights. Biochem J. 2006;398:295-302.

Ricci-Silva ME, Valente RH, León IR, Tambourgi DV, Ramos OHP, Perales J, Chudzinski-Tavassi AM. Immunochemical and proteomic technologies as tools for unravelling toxins involved in envenoming by accidental contact with *Lonomia obliqua* caterpillars. Toxicon. 2008;51(6):1017-28.

Riddiford LM, Palli SR, Hiruma K, Li W, Green J, Hice RH, Wolfgang WJ, Webb BA. Developmental expression, synthesis, and secretion of

insecticyanin by the epidermis of the tobacco hornworm, Manduca sexta. Arch Insect Biochem Physiol. 1990;14:171-90.

Riley CT, Barbeau BK, Keim PS, Kézdy FJ, Heinrikson RL, Law JH. The covalent protein structure of insecticyanin, a blue biliprotein from the hemolymph of the tobacco hornworm, *Manduca sexta* L. J Biol Chem. 1984;259(21):13159-65.

Rocha-Campos AC, Gonçalves LR, Higashi HG, Yamagushi IK, Fernandes I, Oliveira JE, et al. Specific heterologous F(ab')2 antibodies revert blood incoagulability resulting from envenoming by Lonomia obliqua caterpillars. Am J Trop Med Hyg. 2001;64(5-6):283-9.

Romanos M. Advances in the use of *Pichia pastoris* for high-level expression. Curr Opin Biotech. 1995;6:527-33.

Rosenfeld SA, Ross OH, Hillman MC, Corman JI, Dowling RL. Production and purification of human fibroblast collagenase (MMP-1) expressed in the methylotrophic yeast *Pichia pastoris*. Protein Expr Purif. 1996;7(4):423-30.

Ryon J, Bendickson L, Nilsen-Hamilton M. High expression in involuting reproductive tissues of uterocalin/24p3, a lipocalin and acute phase protein. Biochem J. 2002;367:271-277.

Saito H, Shimoda M. Insecticyanin of Agrius convolvuli: purification and characterization of the biliverdin-binding protein from the larval hemolymph. Zoolog Sci. 1997;14(5):777-83.

Saito H. Purification and properties of two blue biliproteins from the larval hemolymph and integument of *Rhodinia fugax* (Lepidoptera: Saturniidae). Insect Biochem Mol Biol. 1998;28(12):995-1005.

Saito H. Purification and characterization of two insecticyanin-type proteins from the larval hemolymph of the Eri-silkworm, *Samia cynthia ricini*. Biochim Biophys Acta. 1998;1380:141-150.

Saito H, Yamada H, Kato Y. Isolation and partial characterization of chromoprotein from the larval hemolymph of the Japanese oak silkworm

(*Antheraea yamamai*). Comp Biochem Physiol B Biochem Mol Biol. 1998; 119(4):625-30.

Sayle RA, Milner-White EJ. RASMOL: biomolecular graphics for all. Trends Biochem Sci. 1995;20(9):374-6.

Schoni R. The use of snake venom-derived compounds for new functional diagnostic test kits in the field of haemostasis. Pathophysiol Haemost Thromb. 2005;34(4-5):234-40.

Schubert D, LaCorbiere M, Esch F. A chick neural retina adhesion and survival molecule is a retinol-binding protein. J Cell Biol. 1986;102:2295-2301.

Schubert D, LaCorbiere M, Esch F. A chick neural retina adhesion and survival molecule is a retinol-binding protein. J Cell Biol. 1986;102(6):2295-301.

Seibert CS, Tanaka-Azevedo AM, Santoro ML, Mackessy SP, Torquato RJS, Lebrun I, et al. Purification of a phospholipase A2 from *Lonomia obliqua* caterpillar bristle extract. Biochem Biophys Res Commun. 2006; 342:1027-33.

Shimamoto S, Yoshida T, Inui T, Gohda K, Kobayashi Y, Fujimori K, Tsurumura T, Aritake K, Urade Y, Ohkubo T. NMR solution structure of lipocalin-type prostaglandin D synthase: evidence for partial overlapping of catalytic pocket and retinoic acid-binding pocket within the central cavity. J Biol Chem. 2007;282(43):31373-9.

Sivaprasadarao A, Findlay JB. Structure-function studies on human retinol-binding protein using site-directed mutagenesis. Biochem J. 1994; 300:437-442.

Silva MB, Schattner M, Ramos CR, Junqueira-de-Azevedo IL, Guarnieri MC, Lazzari MA, et al. A prothrombin activator from *Bothrops erythromelas* (jararaca-da-seca) snake venom: characterization and molecular cloning. Biochem J. 2003;369(1):129-39.

Silva GH, Hyslop S, Cruz-Hölfling MA. *Lonomia obliqua* caterpillar venom increases permeability of the blood–brain barrier in rats. Toxicon. 2004; 44:625-34.

Sok AJ, Czajewska K, Ozyhar A, Kochman M. The structure of the juvenile hormone binding protein gene from *Galleria mellonella*. Biol Chem. 2005; 386(1):1-10.

Sousa MM, do Amaral JB, Guimarães A, Saraiva MJ. Up-regulation of the extracellular matrix remodeling genes, biglycan, neutrophil gelatinase-associated lipocalin, and matrix metalloproteinase-9 in familial amyloid polyneuropathy. FASEB J. 2005;19(1):124-6.

Sun J, Bottomley SP, Kumar S, Bird PI. Recombinant caspase-3 expressed in *Pichia pastoris* is fully activated and kinetically indistinguishable from the native enzyme. Biochem Biophys Res Commun. 1997;238(3):920-4.

Taniike M, Mohri I, Eguchi N, Beuckmann CT, Suzuki K, Urade Y. Perineuronal oligodendrocytes protect against neuronal apoptosis through the production of lipocalin-type prostaglandin D synthase in a genetic demyelinating model. J Neurosci. 2002;22:4885-96.

Thomas L, Crawford DL. Cloning of clustered Streptomyces viridosporus T7A lignocellulose catabolism genes encoding peroxidase and endoglucanase and their extracellular expression in *Pichia pastoris*. Can J Microbiol. 1998;44(4):364-72.

Tong Z, Wu X, Ovcharenko D, Zhu J, Chen CS, Kehrer JP. Neutrophil gelatinase-associated lipocalin as a survival factor. Biochem J. 2005; 391(Pt 2):441-8.

Tuladhar SM, Püntmann VO, Soni M, Punjabi PP, Bogle RG. Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass. J Cardiovasc Pharmacol. 2009;53:261-6.

Turpie AGG. New oral anticoagulants in atrial fibrillation. Eur Heart J. 2007;29:155-65.

Urade Y, Hayaishi O. Biochemical, structural, genetic, physiological, and pathophysiological features of lipocalin-type prostaglandin D synthase. Biochim Biophys Acta. 2000;1482(1-2):259-71.

Vandersall-Nairn AS, Merkle RK, O'Brien K, Oeltmann TN, Moremen KW. Cloning, expression, purification, and characterization of the acid alpha-mannosidase from *Trypanosoma cruzi*. Glycobiology. 1998;8(12):1183-94.

Veiga ABG, Blochtein B, Guimarães JA. Structures involved in production, secretion and injection of the venom produced by the caterpillar *Lonomia obliqua* (Lepidoptera, Saturniidae). Toxicon. 2001;39:1343-51.

Veiga ABG, Pinto AFM, Guimarães JA,. Fibrinogenolytic and procoagulant activities in the hemorrhagic syndrome caused by *Lonomia obliqua* caterpillars. Thromb Res. 2003;111:95–101.

Veiga ABG, Ribeiro JMC, Guimarães JA, Francischetti IMB. A catalog for the transcripts from the venomous structures of the caterpillar *Lonomia obliqua*: identification of the proteins potentially involved in the coagulation disorder and hemorrhagic syndrome. Gene. 2005;355:11-27.

Vulinec K. Collective security: aggregation by insects as a defense. In: Evans DL, Schmidt JO, editors. Insect defenses - adaptive mechanisms and strategies of prey and predators. New York: State University of New York; 1990. p. 251-288.

Wang Y, Geer LY, Chappey C, Kans JA, Bryant SH. Cn3D: sequence and structure views for Entrez. Trends Biochem Sci. 2000a;25(6):300-2.

Wang Y, Addess KJ, Geer L, Madej T, Marchler-Bauer A, Zimmerman D, Bryant SH. MMDB: 3D structure data in Entrez. Nucleic Acids Res. 2000b; 28(1):243-5.

Warkentin TE, Crowther MA. Reversing anticoagulants both old and new. Can J Anaesth. 2002;49(6):S11-25.

Willard L, Ranjan A, Zhang H, Monzavi H, Boyko RF, Sykes BD, Wishart DS. VADAR: a web server for quantitative evaluation of protein structure quality. Nucleic Acids Res. 2003;31(13):3316-9.

Wlian L. Ação de um peptídeo derivado do Lopap em cultura de fibroblastos e em modelo experimental de lesão cutânea em ratos [dissertação]. São Paulo: Universidade Federal de São Paulo; 2009.

Wojnar P, van't Hof W, Merschak P, Lechner M, Redl B. The N-terminal part of recombinant human tear lipocalin/von Ebner's gland protein confers cysteine proteinase inhibition depending on the presence of the entire cystatin-like sequence motifs. Biol Chem. 2001;382:1515-20.

Yamada D, Sekiya F, Morita T. Prothrombin and factor X activator activities in the venoms of viperidae snakes. Toxicon. 1997;35:1581-9.

Yamanaka A, Ito T, Koga D, Sato T, Ochiai M, Endo K. Purification and characterization of biliverdin-binding protein from larval hemolymph of the swallowtail butterfly, *Papilio xuthus* L. Biosci Biotechnol Biochem. 2000; 64(9):1978-81.

Yndestad A, Landrø L, Ueland T, Dahl CP, Flo TH, Vinge LE, et al. Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure. Eur Heart J. 2009;30:1229-36.

Yousefi S, Simon HU. Granulocyte apoptosis: death by a secreted lipocalin? Cell Death Differ. 2002;9(6):595-7.

Yu X, Li Z, Xia X, Fang H, Zhou C, Chen H. Expression and purification of ancrod, an anticoagulant drug, in Pichia pastoris. Protein Expr Purif. 2007;55(2):257-61.

Xiong S, Wang YF, Ren XR, Li B, Zhang MY, Luo Y. Solubility of disulfide-bonded proteins in the cytoplasm of *Escherichia coli* and its "oxidizing" mutant. World J Gastroenterol. 2005;11(7):1077-82.

Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta. 2000;1482:298-307.

Zalewska M, Kochman A, Estève JP, Lopez F, Chaoui K, Susini C, et al. Juvenile hormone binding protein traffic - Interaction with ATP synthase andlipid transfer proteins. Biochim Biophys Acta. 2009;1788(9):1695-705.

Zannin M, Lourenço DM, Motta G, Dalla Costa LR, Grando M, Gamborgi GP, et al. Blood coagulation and fibrinolytic factors in 105 patients with hemorrhagic syndrome caused by accidental contact with *Lonomia obliqua* caterpillar in Santa Catarina, Southern Brazil. Thromb Haemost. 2003;89: 355-64.

Zanotti G, Berni R. Plasma retinol-binding protein: structure and interactions with retinol, retinoids, and transthyretin. Vitam Horm. 2004;69:271-95.

Zhang Y, Lee WH, Gao R, Xiong YL, Wang WY, Zhu SW. Effects of pallas' viper (*Agkistrodon halys pallas*) venom on blood coagulation and characterization of a prothrombin activator. Toxicon. 1998;36(1):143-52.