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Rational development of novel leads from animal secretion based on coagulation and cell targets: 1. *In silico* analysis to explore a peptide derivative as lipocalins' signature

Kerly Fernanda Mesquita Pasqualoto*, Linda Christian Carrijo-Carvalho, Ana Marisa Chudzinski-Tavassi*

Biochemistry and Biophysics Laboratory, Butantan Institute, Sao Paulo, SP, Brazil

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ABSTRACT

Animal venoms and secretions have been screened, in our research group, to discover, identify and isolate peptide molecules active in the mammalian haemostatic system. As result, this kind of research has provided a portfolio of promising drug candidates. These novel recombinant proteins have turned out to be multifunctional molecules, and are currently under different development phases. Lopap from bristles of the *Lonomia obliqua* moth caterpillar, for instance, is a prothrombin activator which belongs to the lipocalin family. It displays serine protease-like activity with procoagulant effect, and also induces cytokine secretion and antiapoptotic pathways in human cultured endothelial cells. Furthermore, a Lopap-derived peptide has showed to induce collagen synthesis in fibroblast culture and in animal dermis. Here, the molecular properties (steric, electronic, hydrophobic, geometric), which are strongly dependent on chemical structure, were investigated by applying chemometric and computational chemistry methods. It was considered different patterns of amino acid substitution related to the lipocalins' motif 2, which was recently shown to modulate cell survival. The calculated molecular properties were generally maintained in all investigated peptides extracted from three-dimensional structures of Protein Data Bank (1t0v, 1bbp, 1kxo, 2hzr, 1iiu, 1jyj, 1gka, 1s44, 3ebw) when compared to Lopap-derived peptide, specially the molecular shape and electronic density distribution, validating the lipocalin sequence signature previously reported. Indeed, those two properties are quite important for the molecular recognition process.

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1. Introduction

The screening of venoms and secretions has been performed, in our research group, to discover, identify, and isolate peptide molecules acting in the mammalian haemostatic system. As result, a portfolio of promising drug candidates has been provided. Among these candidates is a member of the lipocalin family, called Lopap (***L****onomia* ***o****bliqua* **p**rothrombin **a**ctivator **p**rotease), isolated from bristles of *L. obliqua* moth caterpillar (Reis et al., 2001a,b). These recombinant proteins have turned out to be multifunctional molecules and are currently under different development phases. Lopap, for instance, displays serine protease-like activity with procoagulant effect, and also induces cytokine secretion and antiapoptotic pathways in human cultured endothelial cells (Fritzen et al., 2005; Waismam et al., 2009). Furthermore, a Lopap-derived peptide was capable of inducing collagen synthesis in fibroblast culture and animal dermis (Carrijo-Carvalho et al., 2012). The

* Corresponding authors. Labratório de Bioquímica e Biofísica, Instituto Butantan, Av. Vital Brasil 1500, 05503-900 São Paulo, SP, Brazil. Tel.: +55 11 2627 9738; fax: +55 11 2627 9748.

E-mail addresses: kerlypasqualoto@gmail.com (K.F. Mesquita Pasqualoto), amchudzinski@butantan.gov.br (A.M. Chudzinski-Tavassi).

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exploitation of these novel recombinant proteins as well as their derivative peptides increases the chances of developing new pharmaceutical products as radical innovation.

As already mentioned, Lopap belongs to the lipocalin family, and members of this family are found in a wide range of species, with roles in metabolism, coloration, perception, reproduction, growing or development stages, and modulation of immune and inflammatory responses (Flower, 1996; Seppala et al., 2002; Flo et al., 2004; Ganfornina et al., 2005). From the structural point of view, lipocalins are conformationally well conserved β-barrel proteins (Skerra, 2000) sharing three preserved motifs in their amino acid sequence (Chudzinski-Tavassi et al., 2010). Regarding different species, the degree of sequence conservation for a particular lipocalin is rather high. Otherwise, sequence homology among lipocalins with differing biochemical functions is remarkable low, sometimes less than 10% (Cowan et al., 1990), and just a few lipocalins with distinct physiological roles occur within one organism (Skerra, 2000).

Through the application of a peptide mapping approach and tertiary structure comparison, Chudzinski-Tavassi and co-workers (2010) identified a lipocalin sequence signature (YAIGYSC) related to motif 2, which is able to modulate cell survival. The seven amino acids peptide was named pM2c and is located in the G-β-sheet (Flower, 1996) of Lopap three-dimensional (3D) model (see Fig. 1) and related antiapoptotic lipocalins. However, no molecular target or specific interaction was found yet.

Then, before the development of novel hits (*in vitro* activity) and/or leads (*in vitro* and *in vivo* activity) as potential cytoprotective drug candidates, based upon structure–property or structure–activity relationships, our purpose was to theoretically investigate the molecular properties regarding different patterns of amino acid substitution related to the motif 2 of lipocalins by applying chemometric and computational chemistry methods. It is well-known that molecular properties are directly dependent on the chemical/molecular structure, which is in general responsible for the molecular recognition process and, subsequently, biological response or function. In this study, an exploratory data analysis, which comprises hierarchical cluster analysis (HCA) (Beebe et al., 1998; Ferreira et al., 1999; Ferreira, 2002) and principal components analysis (PCA) (Beebe et al., 1998; Ferreira et al., 1999; Ferreira, 2002), was carried out to provide the samples (seven amino acids sequences) classification through either a similarity index or a linear combination of the original data. The findings will be helpful to confirm or not the pM2c sequence as the lipocalins' signature.

2. Methods

2.1. Data set, 3D molecular models, and molecular properties calculation

The choice of data set was based upon the findings from FASTA sequences' alignment. The Lopap monomer sequence was used as reference. The tool Sequence Annotated by Structure (SAS) from European Bioinformatics Institute website (http://www.ebi.ac.uk/thornton-srv/databases/sas/) was employed in this step. SAS uses FASTA to scan a given protein sequence against all the proteins of known 3D structure in the Protein Data Bank (PDB) (www.pdb.org; Berman et al., 2000). The sequences best scored having more than 25% of total identity with Lopap monomer sequence were evaluated, and it was chosen ten different patterns of

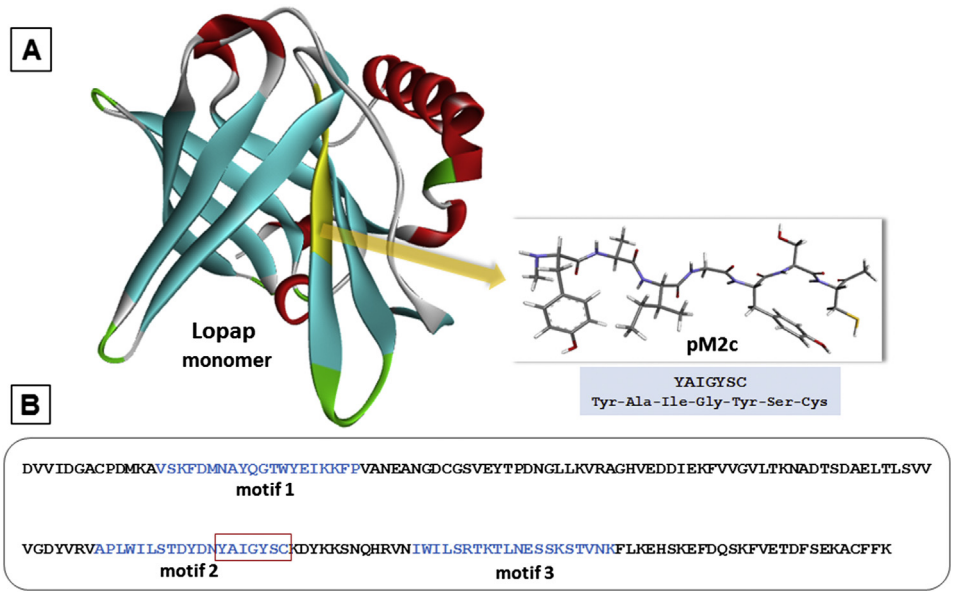

Fig. 1. (A) Ribbon representation of the 3D molecular model of Lopap monomer (β-sheets are presented as flat ribbons in cyan color and α-helices as spiral ribbons in red). The region in G-β-sheet corresponding to the seven amino acid sequence (pM2c) is highlighted in yellow. The 3D molecular model of pM2c extracted from Lopap monomer is displayed as stick model (carbon atoms are in gray, oxygen in red, nitrogen in blue, sulfur in yellow and hydrogen atoms are in white) (Discovery Studio v3.1.1; Accelrys Software Inc., 2005–2011). (B) Amino acids sequence of Lopap showing the three conserved motifs in light blue letters. The lipocalin signature sequence (pM2c) is displayed in a red square. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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