

PAF-acetyl hydrolases from *Rhodnius prolixus*: tissue and temporal expression patterns, and comparison between fat body, hemolymph and salivary gland isoforms.

Côrte-Real,R.<sup>1</sup>, Azambuja,P.,<sup>1,2</sup>, Garcia,E.S.,<sup>1,2</sup>, Genta,F.A.<sup>1,2</sup>  
cortereal@ioc.fiocruz.br

1 – IOC-FIOCRUZ; 2 – INEM-CNPq

The platelet-activating factor (PAF), 1-O-alkyl-2-acetyl-sn-glycero-3-phosphoryl-choline, a natural membrane phospholipid, induces several physiologic responses as phagocytosis, aggregation, adhesion, degranulation and diverse morphological changes in mammals. It is known that the acetyl group at the sn-2 position of its glycerol backbone is required for biological activity and that deacetylation of PAF, which is catalyzed by a plasmatic enzyme, PAF-acetyl hydrolase (PAF-AH; E.C. 3.1.1.47), induces loss of activity. PAF have never been detected in insects, and PAF-AH was only described in salivary glands of fleas. PAF-AH from insects was never characterized. In this work, we described and characterized PAF-AH activities from salivary glands, fat body and hemolymph from *R. prolixus* 5<sup>th</sup> instar nymphs. Using a fluorogenic substrate, 1-palmitoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-sn-glycero-3-phosphocoline, we measured specific activities of 700±100, 4000±600, 160±70, 30±10 U/mg in cell-free hemolymph, fat body, salivary glands and hemocytes homogenates, respectively. Midgut has negligible activity. Fat body PAF-AH (fPAF-AH) peaks 5 days after blood feeding, and cell-free hemolymph PAF-AH (hPAF-AH) has maximum values before feeding or moult. Salivary gland PAF-AH (sPAF-AH) decreases after feeding, recovering initial levels at day 7. Hemocytes PAF-AH peaks at day 5. High pressure gel filtration chromatography (Superdex 200, AKTA) of salivary glands, fat body and hemolymph homogenates results in one major PAF-AH activity with relative molecular weight of 10, 150 and 150kDa, respectively. SDS-PAGE of these activities showed enrichment of a protein with relative molecular weight of 10, 44 and 42kDa, respectively. These data suggest that fPAF-AH and hPAF-AH are the same isoform, different from sPAF-AH. Probably, fPAF-AH and hPAF-AH regulate the insect immune response, being complexed with lipoproteins, and sPAF-AH inhibits vertebrate immune response during insect feeding.

Supported by FAPERJ, CNPq and FIOCRUZ

Keywords: *Rhodnius prolixus*, PAF, PAF-acetyl hydrolase