Exploring the selectivity of the antimicrobial peptide PMAP-23: a fluorescence study on red blood cells.

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The indiscriminate and widespread use of antibiotics has led to the emergence of pathogenic bacteria endowed with multidrug resistance, which are diffusing at an alarming rate. Antimicrobial peptides (AMPs) are considered as promising lead compounds for the development of a new class of antibiotic drugs that could fight resistant bacteria. These peptides constitute a fundamental component of the innate immune defense of many organisms [1], showing activity against a wide range of pathogens. Their mechanism of antibacterial activity is mainly based on association to the pathogen plasma membrane and perturbation of its permeability, through the formation of pores. Since no association to specific receptors or proteins is involved in this process, the insurgence of resistance is a very rare event. A prominent feature of AMPs is their ability to discriminate between mammal and bacterial cells, being toxic only toward pathogen organisms. Selectivity is supposedly determined by the different lipid composition of bacterial and eukaryotic membranes.

Biophysical investigations on peptide-membrane interaction and pore formation are usually carried out using model membranes, but in this case quantitative comparison with microbiological studies on AMP activity on cells is difficult, due to the very different experimental conditions. Recent studies conducted in our lab on a dansyl-labelled analogue of PMAP-23, a natural, cationic and amphipathic antimicrobial peptide belonging to the cathelicidin family, provided for the first time a direct determination of the minimal number of membrane-bound peptide molecules which are necessary to kill a bacterial cell (*E. coli*). We determined that a high membrane coverage by the peptide is required to display its antibacterial activity [2].

In order to extend our previous results to mammalian cells, we performed under exactly the same experimental conditions an assay of haemolytic activity and a determination of peptide association to RBCs (red blood cells). RBCs were purified from human blood with repeated washing and centrifugation steps, and then dispersed in a NaCl 0.9%, HEPES 5mM, pH 7.3 buffer. The hemolysis data (Figure 1) indicate that under the conditions employed in our experiment, cell lysis starts at a total peptide concentration of 1 µM. The fraction of dansylated PMAP-23 molecules which are not associated to the RBCs was determined by measuring the fluorescence intensity emitted by the peptides remaining in the supernatant after centrifugation of the sample (Figure 2). The results of repeated independent experiments (properly corrected for inner-filter effects due to the absorption of released haemoglobin) showed that 44 ± 8 % of total peptide binds to RBCs, at 1 µM total peptide concentration and 4.5 x 10^7 cells/ml. These data correspond to 6 millions of bound peptide molecules per cell. Considering an outer surface of $1.4\,10^8$ nm^2 for an erythrocyte [3] and an area of at least 5 nm^2 for a single bound peptide molecule [2], our data indicate that membrane perturbation starts taking place when the peptides cover more than 20% of the RBC surface.

In summary, our preliminary results indicate that a high coverage of RBC surface by the peptide is required to cause membrane perturbation and hemolysis. Comparable results have been previously obtained with bacteria and model membranes [3]. Therefore, selectivity of dansylated PMAP-23 apparently is not due to its behaviour once bound to the cellular membranes, which is similar in widely different systems. Other phenomena, such as preferential binding to one cell type, must be considered to explain the selectivity of AMPs.

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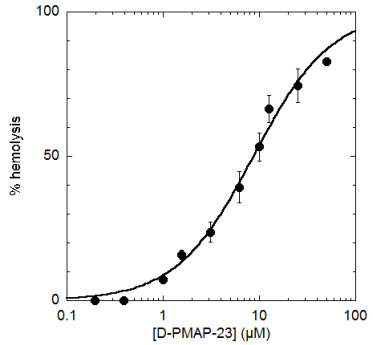

Fig. 1: Percentage of RBC hemolysis caused by dansylated PMAP-23 at different concentrations ([RBCs]=4.5 x 10^7 cells/ml; 2h incubation).

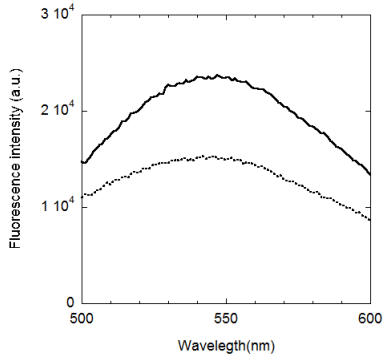

Fig. 2: Representative fluorescence spectra of dansylated PMAP-23 (1 µM) before (solid line) and after (dotted line) incubation with RBCs. λ_{exc} = 340 nm.