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Self-defensive coatings for ultimate antibiotics degradation: Fast elaboration of immobilized enzymes process for water treatment

Sebastien Bonot, Rodolphe Mauchauffe, Nicolas Boscher and **Henry-Michel Cauchie**
Luxembourg Institute of Science and Technology, Luxembourg

Due to their intensive use, antibiotics are now ubiquitous in water. They are present in sub-inhibitory concentrations and can then induce the emergence of antibiotic-resistant bacteria and bacterial ecosystem imbalances. The current water treatments are not efficient for the antibiotic elimination and can generate toxic secondary metabolites. The objective of our work is to develop enzymatic processes enhancing the antibiotics degradation with generation of non-toxic byproducts. To this end, enzymes encoded by antibiotic resistance genes (a β-lactamase and a New Delhi metallo-β-lactamase are known to degrade xenobiotics (laccase) were selected. These enzymes were covalently immobilized on biochips M Kaldnes (used in moving bed reactors) coated with either an epoxy or a quinone-rich film obtained by plasma deposition and self-polymerization of dopamine. Immobilized protein quantities varied from 12 to 95 μg cm^{-2} and enzymatic activities were observed up to 576 hours compared to a maximum of 48 hours for the free enzymes. The degraded antibiotic concentrations were 2-15 folds higher with immobilized enzymes. The erosion tests showed that after exposure to a high water flow, the enzymes are still immobilized and active. Further, the generated metabolites show no acute toxicity (Microtox, tests on intestinal cells, Daphnia and algae ISO tests). The surface saturation with Tween 20 efficiently prevented microorganism adhesion and showed an unexpected advantage: The extension of the enzymatic activity. When the surfaces become inactive a simple protocol enables to remove the enzymes, to reload the biochips with new active enzymes and then to reach the original degradation efficiency.

sebastien.bonot@free.fr

Unraveling the mechanisms of hBD3 internalization and trafficking in the A549 epithelial airway type of cells: A key role for the complex CD98 membrane protein

Ersilia Nigro
CEINGE-Biotecnologie Avanzate, Italy

Human beta defensins (hBDs) are small cationic peptides that play a pivotal role in the innate immune response. They exert direct antimicrobial activity against a wide spectrum of bacteria and viruses; their expression is up-regulated during infection and inflammation. All hBDs are secreted by epithelia, which represent their natural site of action. However, little is known about their interaction with human epithelial cells. Here, we used a functional proteomic approach to search for cell surface receptors that could play a role in the interaction and internalization of hBD3 in A549 cells. Out of the proteins identified through this analysis we focused on CD98, a type II transmembrane protein involved in amino acid transport, cell adhesion, inflammation, immune response and attachment of enteric bacterial pathogens. Through confocal microscopy we found that CD98 and hBD3 extensively colocalize on the basolateral domain of A549 cells. We then confirmed their direct binding by fluorescence resonance energy transfer and surface Plasmon resonance and mapped the region of interaction to residues 304-414 of CD98. Finally, we found that knockdown of CD98 expression in A549 cells impairs both hBD3 cell surface binding and internalization and conversely treatment of A549 cells with hBD3 dramatically reduces the expression of CD98. Overall, these data provide evidence that CD98 plays a specific role in hBD3 membrane trafficking, assisting the ready entry and internalization of the peptide in epithelial cells. The interaction of hBD3 with CD98 and in particular the down-regulation of its expression might have a functional role in the activity of hBD3.

nigro@ceinge.unina.it