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Photosensitizing proteins for a targeted antibacterial photodynamic inactivation

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Summary. — One of the emerging global health threats is the drug-resistant microbial diseases that could cause millions of deaths each year by 2050. A very promising tool to tackle antimicrobial resistance is photodynamic therapy that exploits the ability of a photosensitizer to generate oxidizing species upon illumination to kill bacteria. The research activity in this field is very active and, following this approach, we aim to develop a targeted theranostic agent where a protein is used as a "modular" carrier, transporting a fluorescent photosensitizer, and an antibody to confer selectivity to the photodynamic effect.

1. – Introduction

One of the priorities of WHO is the fight against antimicrobial resistance, as demonstrated by a WHO's global campaign, started in 2015, to raise awareness of this emergency among the general public, health workers and policy makers. In 2016 the British government commissioned the British Minister of Commerce, Lord Jim O'Neill, a well-known economist, the analysis of the problem of antibiotic resistance, in order to propose feasible solutions on a global scale. In the resulting report, Lord Jim O'Neill affirmed that the damage to human health represented by antibiotic resistance is much more worrying than the 2008 financial crisis [1].

Antibiotics are an essential tool against infections: surgical operations could not be performed without the aid of these drugs. For this reason, excessive and inappropriate use limits their effectiveness.

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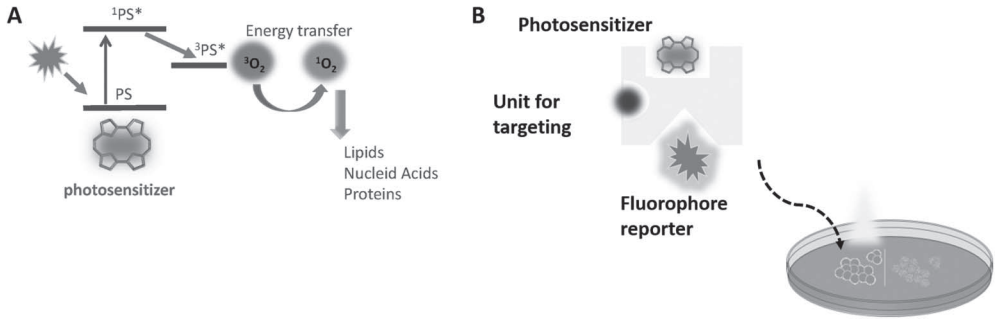

Fig. 1. – (A) Simplified Jablonski diagram for the photosensitization of molecular oxygen by a photosensitizer (PS). PS, ^{1}PS* and ^{3}PS* denote respectively ground, excited singlet and triplet state of the photosensitizer. Absorption of a visible light photon, and intersystem crossing are indicated by a vertical black and a gray arrow (from ^{1}PS* to ^{3}PS*), respectively. The singlet oxygen ($^1O_2$) resulting from a Dexter-type energy transfer mechanism is able to damage the fundamental cellular constituents. (B) Pictorial scheme of a targeted aPDT treatment of bacteria with a "modular" self-assembling theranostic construct, as proposed in our labs.

The development of alternative strategies for preventing and treating infectious diseases of bacterial origin is therefore of great interest [2, 3]. Among them, antimicrobial photodynamic therapy (aPDT) is a promising methodology that does not induce resistance and exploits the photodynamic effect (fig. 1(A)), which consists in the use of otherwise non-toxic molecules, named photosensitizers (PSs), and visible light in the presence of molecular oxygen, to produce reactive oxygen species, mostly singlet oxygen, which result in cellular toxicity [4-8].

2. – Strategies for a targeted aPDT

An effective PS to be used in aPDT should combine targeting, therapeutic and (possibly) diagnostic functions within a single nanoscale complex [9].

There are four relevant issues to be addressed in aPDT: i) the insolubility of standard PSs in aqueous solutions; ii) the limited lifetime of singlet oxygen that requires the photosensitization process to occur within 100 nm from the target [10]; iii) the difficulty to penetrate the Gram negative cell wall, precluding the contact of PS with the inner cell membrane; iv) the selectivity of the photodynamic action.

The first two issues have been addressed in the literature using "classical" drug delivery [11]. The different strategies to address the third and the fourth points have been recently reviewed [12].

The simplest strategy consists in exploiting the negative charge on bacterial cell walls, favoring the formation of electrostatic interactions between the walls and cationic PSs [13] or PSs conjugated to poly-cationic materials, like basic amino acids [14] or conjugating antibiotics [15].

The strategy in general is to develop constructs where the PS is conjugated to "passively" targeting poly-cationic or antimicrobial materials, or to "actively" targeting pathogen-specific antimicrobial peptides, or antibiotics [12], possibly introducing a spacer [16], or using macro- and nano-PS [17]. The most common macro- or supramolecular vehicles for the photoactivatable pro-drug that are able to increase cellular uptake (actively or passively) are reported below.

Micelles and liposomes: in particular, cationic liposomes represent an interesting tool, due to the fact that they can load PSs and target the bacterial membranes through

electrostatic interactions [18]. A possible disadvantage of this approach is that these constructs can also ruin the integrity of eukaryotic cell membranes.

Oligosaccharides: there are numerous examples of nano-PS that take advantage of the self-assembling properties of oligosaccharides, like chitosan [19] or cyclodextrins. They encapsulate or vehicle [20] the PS molecule covalently [21] or non-covalently [22], and can be also functionalized with targeting units, such as antimicrobial peptides.

Polymers and nanoparticles: hyperbranched macromolecules, dendrimers, carbon nanoparticles or nanodots are interesting tools to conjugate or encapsulate [23] photoactive molecules and targeting units [24]. Moreover, gold and silica nanoparticles represent well known nanocarriers for PS delivery and provide a surface that can be functionalized to confer targeting properties to the construct. However, another approach has been suggested, that consists in using nanomaterials with an antimicrobial activity, such as silver nanoclusters [25] that release Ag^+ ions and allow to extend the antibacterial activity even after the photosensitization process or core-shell silver-silica nanoparticles that take advantage of the plasmonic effect to enhance the production of singlet oxygen [26].

Immunoconjugates and protein conjugates: interestingly, antibodies and proteins can be used to develop supramolecular structures combining the capacity of PS loading and bacteria targeting. For example, chlorine e6 was conjugated to a polyclonal antibody to *S. aureus* and to a penicillin-binding protein 2a monoclonal antibody [12] to treat methicillin-resistant *S. aureus* infections of blood [27].

3. – Perspectives for further developments using protein transporters

Among the above outlined strategies to assemble effective PS for aPDT, the use of proteins appears particularly promising. Besides being obviously a biocompatible material, proteins constitute a versatile and modular platform to merge different functions in a single supramolecular structure. We have recently shown that proteins, *e.g.*, apo-myoglobin or β-lactoglobulin, can be exploited as passive carriers to transport non-covalently bound hydrophobic PSs to cellular structures [28-37]. Although these systems provide an efficient delivery by preventing PS aggregation, they are devoid of targeting capability.

However, chimeric proteins can be engineered to comprise different domains with specific functions, which in addition to the PS transport [38], include the capability of recognizing specific molecular structures on target cells. An example of such modular system is based on the tetrameric protein streptavidin from *Streptomyces avidinii*, which provides four biotin binding sites to which biotinylated proteins with targeting capability (*e.g.* antibodies) and PSs derivatives can be anchored [39]. Further flexibility to this system is provided by the possibility of covalently labeling streptavidin with a fluorescent dye, thus achieving a theranostic assembly that can be tailored to specific applications by replacing the targeting unit. As an example, we propose to address infections by *Staphylococcus aureus*, exploiting the known interactions of Immunoglobulin G with protein A on *S. aureus*, driving a streptavidin based supramolecular construct with photosensitizing properties to the bacterial surface (fig. 1(B)) [40].

In conclusion, in the last decades, the antimicrobial photodynamic approach has demonstrated its efficacy and opens promising prospects against multidrug-resistance strains of bacteria. In particular, we believe that a methodology based on protein-based carriers can guarantee the necessary biocompatibility, absence of toxicity, and can provide also a modular platform for combining different agents, principally for the therapy, the imaging, and for conferring specificity, and, if possible, to monitor the efficacy of treatment in time, after administration of PS and illumination.

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REFERENCES

[1] O'Neill Jim, *Tackling drug-resistant infections globally: final report and recommandations. Review on Antimicrobial Resistance* (Government of the United Kingdom, London, UK), (2016) pp. 1–76.
[2] Aminov R. I., *Front. Microbiol.*, **1** (2010) 134.
[3] Ventola C. L., *Pharmacol. Ther.*, **40** (2015) 277.
[4] Dolmans D. E. J.G. J. *et al.*, *Nat. Rev. Cancer*, **3** (2003) 380.
[5] Michl J., in *Handbook of Photochemistry*, edited by Montalti M., Credi A., Prodi L. and Gandolfi M. T., III edition (CRC Press Taylor & Francis Group, Boca Raton) 2006, pp. 1–47.
[6] Cadet J., *et al.*, in *Singlet Oxygen: Applications in Biosciences and Nanosciences*, edited by Nonell S. and Flors C. (Royal Society of Chemistry) 2016, pp. 393–407.
[7] Girotti A. W. *et al.*, in *Singlet Oxygen: Applications in Biosciences and Nanosciences*, edited by Nonell S. and Flors C. (Royal Society of Chemistry) 2016, pp. 409–430.
[8] Fudickar W. *et al.*, in *Singlet Oxygen: Applications in Biosciences and Nanosciences*, edited by Nonell S. and Flors C. (Royal Society of Chemistry) 2016, pp. 431–446.
[9] Bardhan R., *et al.*, *Acc. Chem. Res.*, **44** (2011) 936.
[10] Ogilby R., *Chem. Soc. Rev.*, **39** (2010) 3181.
[11] Lammers T. *et al.*, *Acc. Chem. Res.*, **44** (2011) 1029.
[12] Klausen M. *et al.*, *Molecules*, **25** (2020) 5239.
[13] Felgenträger A. *et al.*, *BioMed Res. Int.*, **2013** (2013) 482167.
[14] Sahu K. *et al.*, *Lasers Med. Sci.*, **28** (2012) 465.
[15] Nieves I. *et al.*, *Bioorgan. Chem.*, **97** (2020) 103661.
[16] Ucuncu M. *et al.*, *Chem. Commun.*, **56** (2020) 3757.
[17] Maldonado-Carmona N. *et al.*, *Photochem. Photobiol. Sci.*, **19** (2020) 445.
[18] Boccalini G. *et al.*, *J. Mater. Chem. B.*, **5** (2017) 2788.
[19] Helander I. *et al.*, *Int. J. Food Microbiol.*, **71** (2001) 235.
[20] Pourhajibagher M. *et al.*, *Front. Phys.*, **6** (2018) 6.
[21] Ribeiro C.P. *et al.*, *Photodiagn. Photodyn. Ther.*, **31** (2020) 101788.
[22] Castriciano M. A. *et al.*, *Biomacromolecules*, **18** (2017) 1134.
[23] Staegemann M. H. *et al.*, *Chem. Eur. J.*, **23** (2017) 3918.
[24] Gao Z. *et al.*, *Anal. Bioanal. Chem.*, **412** (2020) 871.
[25] Shitomi K. *et al.*, *Photodiagn. Photodyn. Ther.*, **30** (2020) 101647.
[26] Planas O. *et al.*, *J. Am. Chem. Soc.*, **138** (2016) 2762.
[27] Kim G. *et al.*, *Photochem. Photobiol.*, **94** (2018) 744.
[28] Hally C. *et al.*, *Trans. Biophoton.*, **2** (2020) e201900031.
[29] Comas-Barceló J. *et al.*, *RSC Adv.*, **3** (2013) 17874.
[30] Delcanale, P. *et al.*, *Sci. Rep.*, **5** (2015) 15564.
[31] Rodríguez-Amigo B. *et al.*, *J. Dairy Sci.*, **98** (2015) 89.
[32] Delcanale P. *et al.*, *J. Agric. Food Chem.*, **64** (2016) 8633.
[33] Galstyan A. *et al.*, *Chem. Eur. J.*, **22** (2016) 5243.
[34] Delcanale P. *et al.*, *J. Matter Chem. B*, **5** (2017) 1633.
[35] Pezzuoli D. *et al.*, *Food Control*, **94** (2018) 254.
[36] Abbruzzetti S., *Nuovo Cimento C*, **41** (2018) 116.
[37] Delcanale P. *et al.*, *Photochem. Photobiol. Sci.*, **19** (2020) 324.
[38] Giuntini F. *et al.*, *Photochem. Photobiol. Sci.*, **10** (2011) 759.
[39] Eng M. S. *et al.*, *Photochem. Photobiol. Sci.*, **17** (2018) 539.
[40] Sause W. E. *et al.*, *Trends Pharmacol. Sci.*, **37** (2016) 231.