Polymeric nanoreactors: a new way to improve antioxidant therapy

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Many naturally occurring proteins offer promise, particularly in diseases caused by insufficient amounts or inactive variants of those proteins. Unfortunately, this promise has not yet been fulfilled (except insulin and growth factor), largely because of significant barriers to effective bioavailability, as it is the case for antioxidant enzymes, such as superoxide dismutase (SOD), catalase, peroxidases. Even if a variety of approaches have been examined to address these bioavailability limitations, including the synthesis of protein mimics, modified proteins or encapsulation in liposomes/polymeric carriers from where they are delivered, these approaches have various drawbacks, such as instability, loose of activity during the modification, or short circulation lifetime, and release of the compound in non-controlled conditions/biological compartments. Nanoreactors based on encapsulation of active compounds in polymersomes that enable them to act *in situ,* have recently been developed as a new way for their protection.

Here we present the concept of antioxidant nanoreactors based on the encapsulation of SOD/SOD-mimics in polymersomes with oxigen permeable membrane. Superoxide anions ($O_2^{\cdot -}$) represent one of the major reactive oxygen species involved in oxidative stress in cells, and known to play an important role in diseases such as asthma, atherosclerosis, neurodegenerative diseases, cancer and AIDS. The antioxidant nanoreactors we develop serve to protect the antioxidant agents and simultaneously to let them act *in situ*.

First, the antioxidant nanoreactor was formed by the encapsulation of SOD in amphiphilic copolymer vesicles made of poly-(2-methyloxazoline)-poly (dimethylsiloxane)-poly (2-methyloxazoline).[1] We choose amphiphilic block copolymers because their self-assembly as vesicles allow the simultaneous encapsulation of the active compound in mild conditions that are supposed to not afect the sensitive enzyme. In a second approach, we optimized the nanoreactors by encapsulating low molecular weight SOD-mimics that are easier to be encapsulated and with an increased encapsulation efficiency.[2] Encapsulated SOD/SOD-mimics were characterised by spectroscopic and paramagnetic resonance techniques and we established that the metal binding region, which represents the catalytic site was not affected by the encapsulation procedure. The function of these antioxidant nanoreactors was tested by pulse radiolysis, which demonstrated that the compound preserved its activity inside the cavity of polymersomes. We developed the antioxidant nanoreactor by changing changing the procedure of vesicles formation or by modifying the molecular properties of the block copolymers, in respect with the hydrophobic/hydrophilic domains, vesicles sizes and oxygen permeability of the membranes. In addition we could demonstrate that the antioxidant nanoreactors based on SOD/SOD-mimics have no cytotoxic effects on monocyte cells. This emphasizes the capacity of such nanoreactors in antioxidant therapy.

Simple and robust, antioxidant nanoreactors represent a new strategy to cope with the necessity to increase the concentration of the antioxidant agent at biological sites: the protein/mimic acts inside the nanoreactor with the membrane acting as a shield, and no release is required in order to obtain the biological effect. This represents a new direction for developing drug delivery applications in oxidative stress, that avoids the disadvantages of conventional drug release carriers.

References:

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[2] V. Balasubramanian, O. Onaca, F. Axthelm, D. Hughes, M. Grzelakowski, W. Meier, C. G. Palivan, *J. Optoelectronics & Adv. Mat*., **1(6)**, 2009, 1083.