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Phage display-selected peptides for binding and synthesis of nanoparticles: ZnO as an example

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Abstract

Nanoparticles of metal oxides are widely used in bionanotechnology, particularly in bio-medical applications; e.g., construction of biosensors, separation of biological materials, molecular imaging, and anticancer and antimicrobial therapies. However, synthesis of these nanoparticles using physico-chemical methods is problematic, because such procedures require high-temperature processes and harsh chemical treatments. The use of peptides specifically binding particular nanoparticles or nanostructures and facilitating their synthesis appears to be an encouraging alternative. Specific peptides capable of such reactions may be identified with the use of the phage display method. In this mini-review, zinc oxide is discussed as an exemple material whose nanoparticles can be bound and synthesized by such peptides exposed on the surface of bacteriophage capsids. An analysis of reports on studies into methods of peptide-aided synthesis of ZnO nanoparticles has indicated that, despite the encouraging results obtained so far, further studies are necessary to optimize such procedures. This may also be true for nanoparticles of other materials, particularly metal oxides.

Key words: zinc oxide, ZnO nanoparticles, ZnO-binding peptides, phage display

Introduction: nanomaterials and bionanotechnology

Nanotechnology is a rapidly developing field based on the use of particles of various materials whose size, in at least one dimension, is between 1 and 100 nm. Nano-sized materials often have physical and chemical properties that are substantially different from the same materials at larger scales. Useful in diverse fields such as physics and chemistry, nanomaterials appear to be principally applicable in biology and medicine (Whitesides, 2003). Employment of nanomaterials in bio-medical applications (such as construction of biosensors, separation of biological materials, molecular imaging, anticancer and antimicrobial therapies, and others), as well as the use of biological systems to synthesize or isolate nanoparticles and nanostructures, is referred to as bionanotechnology (de Morais et al., 2014; Schrofel et al., 2014).

The applications of nanotechnology are extremely wide-ranging and include microelectronics, textiles, energy production, novel therapies and diagnostic tools, or new generation cosmetics (McIntyre, 2012). On the other hand, while initial works had suggested that nanomaterials are generally safe for most organisms, including humans, more recent studies have signaled the possible toxicity of at least some nanoparticles (Wiesenthal et al., 2011; Win-Shwe and Fujimaki, 2011; Nohynek and Dufour, 2012; Wang et al., 2013; Migliore et al., 2015). The aim of this review article is not, however, to discuss the whole field of nanotechnology or bionanotechnology or their advances and limitations. Rather, we will focus on a single nanomaterial, zinc oxide, and will review the progress in the synthesis of ZnO nanoparticles with the use of peptide sequences, identified as a bio-template by a biological system called phage display. This, however, can be treated as an example of bionanotechnological applications, and the indicated implications may be considered in a broader context.

Zinc oxide as one of the elite nanomaterials

Zinc oxide is one of most widely used nanomaterials. Recently, it has been included within the elite group of

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nanomaterials, together with graphene, carbon nanotubes and gold (Kumar et al., 2015). Of particular interest is the use of ZnO nanoparticles in the development of novel biosensors (Yakimova et al., 2012), bioimaging, and drug delivery (Xiong, 2013). In fact, ZnO reveals favorable pharmacokinetic features, though detailed parameters depend on several factors, such as particle size, surface charge, surface coating, protein binding, exposure route and dose (Lin et al., 2015). As zinc oxide nanoparticles can act as effective filters of UV light, they are employed as compounds in modern cosmetics, particularly those used as sunscreens (Loh and Dunn, 2012). In the era of the antibiotic crisis (Hansen et al., 2015), there is a new hope that bacterial infections will be combated with the use of ZnO nanoparticles. Such nano-sized materials have been found to possess a potent antibacterial activity, and since they are produced as artificial bodies, it is hoped that development of resistance to them will not be as obvious as it is to natural compounds (Pelgrift and Friedman, 2013). It appears that the size of ZnO particles, which is within nanoscale, is crucial for their efficient antibacterial activity (Dizaj et al., 2014). One mechanism of their antibacterial activity is the induction of reactive oxygen species formation (Shi et al., 2014), enhanced after UV light absorption (Lipovsky et al., 2013).

Apart from the many encouraging or even enthusiastic reports indicating the wide-ranging applicability of ZnO nanoparticles, as well as early reports suggesting their safety (summarized by Nohynek et al., 2007), more recent studies have revealed that this metal oxide, like other similar compounds, may exert toxicity to eukaryotic cells when acting in the nanoscale size range, as opposed to bulk ZnO powder (Reddy et al., 2007). Among eukaryotes, algae have been found to be the most sensitive organisms – crustaceans and fish being less susceptible – and toxicity to mammalian cells has been proved to be the lowest, although still considerable (Bondarenko et al., 2013). These differences are likely due to organism-specific uptake of nanoparticles by cells, and different efficiencies in oxidative stress induction (Ivask et al., 2014). In the light of the potential toxicity of ZnO nanoparticles to humans, the most worrisome are those reports indicating that such particles may impair the immune response (Roy et al., 2015) and provoke neurodegeneration (Migliore et al., 2015).

Since the use of ZnO nanoparticles brings both new hope for bionanotechnology and some apprehensions regarding their safety, it is clear that further extensive studies on these compounds are necessary. To this end, efficient methods for their synthesis and isolation are indispensable.

Methods for the synthesis and isolation of ZnO nanoparticles

There are various physico-chemical methods to produce ZnO nanostructures, including methods based on the sol-gel route (Tokumoto et al., 2003) or a simple solution route (Yu et al., 2007), sonochemical synthesis (Yu et al., 2015), hydrothermal synthesis (Chen et al., 2007), microwave-assisted synthesis in benzyl alcohol (Bilecka et al., 2009), self-propagating high-temperature synthesis (Hwang et al., 2009), and the growth of crystals in molten hydrous alkali solutions (Zhang et al., 2011). However, such methods are sometimes either not sufficiently efficient or too expensive, or they suffer from contamination with toxic chemicals used in the technological processes. Furthermore, ZnO nanoparticles obtained as a final product may display low stability or undergo the processes of self-aggregation. Therefore, alternative methods based on the use of biological systems are currently considered an eco-friendly, cost-effective, and contamination-free alternative (Żelechowska, 2014; Hussain et al., 2015).

In the simplest of the systems, biological materials have been proposed to facilitate ZnO nanoparticle synthesis (Żelechowska, 2014; Madhumitha et al., 2016). Examples include the use of collagen (Bai et al., 2009), bacteriophage λ DNA (Atanasova et al., 2009), orange juice (Jha et al., 2011), *Ocimum basilicum* (Salam et al., 2014), *Sargassum myriocystum* (Nagarajan and Kuppusamy, 2013), or various microbes (Jayaseelan et al., 2012; Hulkoti and Taranath, 2014). More advanced biotechnological applications appear to be more specific, reproducible and potentially useful in technological processes. In particular, the discovery that various materials of non-biological origin, such as metals and their derivatives, can be specifically bound by peptides of characteristic sequences has allowed researchers to develop methods which employ genetic engineering and biochemical techniques for material fabrication approaches (Whaley et al., 2000). The phage display method, originally introduced by Smith (1985), seems to be the best choice for the identification of peptide sequences for such purposes. This method is based on the construc-

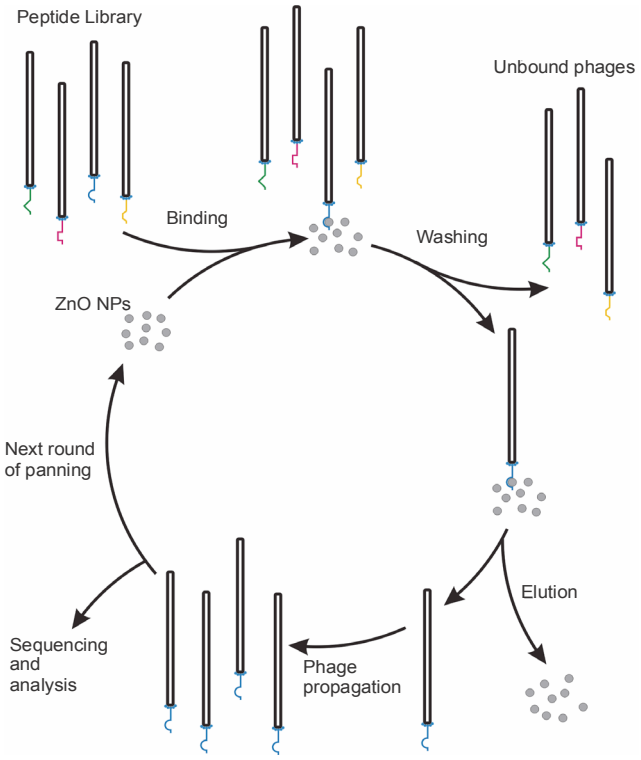

Fig. 1. Scheme for the identification and isolation of bacteriophages binding ZnO nanoparticles (ZnO NPs) due to exposition of specific peptides on capsids

tion of recombinant bacteriophages (i.e. viruses infecting bacterial cells) which bear one of the genes coding for capsid proteins fused to a foreign DNA fragment encoding a peptide of a specific sequence. As a result, after propagation of the recombinant bacteriophage (phage) in the host bacterial cells, such a peptide is exposed on the virion (i.e. phage particle) surface. When a genetic library composed of bacteriophages bearing a battery of different DNA fragments, coding for various peptides and fused to the capsid protein-encoding gene, is constructed, a vast number of peptide sequences can be screened for particular purposes, including the binding of metal oxides or the synthesis of nanoparticles (Eldridge and Weiss, 2015). A scheme for the identification and isolation of recombinant bacteriophages, exposing (on their surfaces) peptides capable of binding particular materials (e.g. ZnO nanoparticles), is presented in Fig. 1.

Phage display-aided selection of ZnO-binding peptides and synthesis of nanoparticles

In the first study devoted to isolating ZnO-binding peptides, a library of random peptides was not exposed on bacteriophage capsids, but on bacterial fimbriae – FimH (Kjaergaard et al., 2000). This approach led to the identification of several ZnO-binding peptides, of which RSNTRMTAROHRSANHKSTQRARS had the strongest affinity and specificity for ZnO. In fact, based on this peptide, subsequent studies aimed at exposing the zinc oxide-binding protein on the surface of a baculoviral capsid (Song et al., 2010a) for detection of chemical composition defects and crystallographic state defects (Vreuls et al., 2010), and the synthesis of ZnO nanoparticles (Song et al., 2010b).

The concept of using ZnO-binding peptides to produce nanoparticles had, in fact, been proposed earlier by Umetsu et al. (2005). With the use of bacteriophages displaying random 12-mer peptides fused to a minor coat protein (pIII) of the M13 phage, this group identified a ZnO-binding peptide (EAHVMHKVAPRP) and demonstrated room-temperature immobilization and mineralization of zinc oxide. Intriguingly, in their approach, the authors finally used a modified, chemically synthesized ZnO-binding peptide to which a glycine linker (GGGS) and a cysteine residue at the C-terminus had been added, facilitating the homogeneous creation of highly-ordered, flower-like structures composed of ZnO nanoparticles with diameters of about 10 nm (Umetsu et al., 2005). In 2011, the same group observed morphology changes in ZnO structures when a ZnO-binding peptide was used in the presence of dipeptides (M-H or H-K) for ZnO NP synthesis (Togashi et al., 2011).

Umetsu et al. (2005) was followed by other studies aimed at synthesizing ZnO nanoparticles in reactions facilitated by a peptide identified with the use of a phage display technique. Tomczak et al. (2009) reported the growth of ZnO hexagonal nanocrystals in the presence of a chemically synthesized peptide (GLHVMHKVAPPR) conjugated with the GGGSC tail and selected with the use of the phage display method. The authors used $Zn(NO_3)_2$ and 1,3-hexamethylenetetramine (HMTA) as precursors in the $Zn(NO_3)_2 \cdot 6H_2O$-HTMA system, and carried out the reaction at 65 °C for three days. Interestingly, by altering the concentration of the peptide, ZnO nanocrystal morphology could be tailored. This was done by decreasing concentrations of the ZnO-binding peptide which resulted in the elongation of ZnO nanoparticles, while their hexagonal character was retained (Tomczak et al., 2009).

Based on peptide sequences identified by Umetsu et al. (2005) and Tomczak et al. (2009), the Perry re-

Table 1. Sequences of ZnO-binding peptides

Amino acid sequence	Reference
RSNTRMTAROHRSANHKSTQRARS	Kjaergaard et al. (2000) Song et al. (2010a) Song et al. (2010b)
EAHVMHKVAPRP	Umetsu et al. (2005)
QNTATAVSRLSP	Umetsu et al. (2005)
ATHTNQTHALYR	Umetsu et al. (2005)
VSNHKALDYPTR	Umetsu et al. (2005)
DSGRYSMTNHYS	Umetsu et al. (2005)
GLHVMHKVAPPR	Tomczak et al. (2009)
VRTRDDARTHRK	Vreuls et al. (2010)
HVNLHS	Okochi et al. (2010a)
RCARRY	Okochi et al. (2010a)
HYQSNW	Okochi et al. (2010a)
HWFHPR	Okochi et al. (2010a)
GAMHLPWHMGTL	Wei et al. (2011)
TMGANLGLESPE	Golec et al. (2012)
TMGANLGLKWPV	Golec et al. (2012)
HSXXH	Rothenstein et al. (2012)
VPGAAEHT	Moon et al. (2015)

group carried out $Zn(NO_3)_2 \cdot 6H_2O$-HTMA-dependent synthesis of ZnO structures with modified morphology (Liang et al., 2011; Limo et al., 2015; Sola-Rabada et al., 2015). An attempt to obtain ZnO nanoparticles at room temperature using another phage-display selected peptide was reported subsequently (Wei et al., 2011). Recently, Moon et al. (2015) employed a M13 phage display library with a 8-mer peptide fused to a pVIII phage coat protein. They carried out a synthesis of micrometer ZnO structures with the use of the $Zn(NO_3)_2 \cdot 6H_2O$-HTMA system and the ZnO-binding peptide bearing a GGGSC linker (Moon et al., 2015).

There have been studies aimed at determining the specific amino acid residues responsible for direct interactions with ZnO. In the case of EAHVMHKVAPRP, ZnO-binding peptide (Umetsu et al., 2005) sequences essential for direct contact with this material were identified as HVMHKV and HKVAPR (Okochi et al., 2010b). The same research group fused a recombinant green fluorescent protein (GFP) to a ZnO-binding peptide, which allowed the identification of a "hot spot" (EAHVMHK) with the highest affinity for ZnO (Yokoo et al., 2010). Additionally, based on these results, 420 chemically synthesized random 6-mer peptides have been screened to isolate specific ZnO-binding peptides (Okochi et al., 2010a), revealing four alternating sequences: HVNLHS, RCARRY, HYQSNW and HWFHPR, which display a slightly weaker affinity for ZnO. Even more advanced investigations have led to the determination of the HSXXH sequence responsible for specific binding of ZnO (Rothenstein et al., 2012). However, both the previously mentioned reports (Kjaergaard et al., 2000; Umetsu et al., 2005; Tomczak et al., 2009; Okochi et al., 2010a; Okochi et al., 2010b; Wei et al., 2011), as well as a study demonstrating a phage display-facilitated isolation of novel peptides capable of binding ZnO and synthesizing nanoparticles of this material (Golec et al., 2012), indicated that the HSXXH motif is not necessary for efficient ZnO binding (Table 1). On the contrary, it seems that there is a huge variability of peptide sequences that can efficiently bind ZnO. Moreover, some peptides can themselves be used for an effective synthesis of ZnO nanoparticles. Thus, it has been proposed that the distinct properties of particular ZnO-binding peptides may be

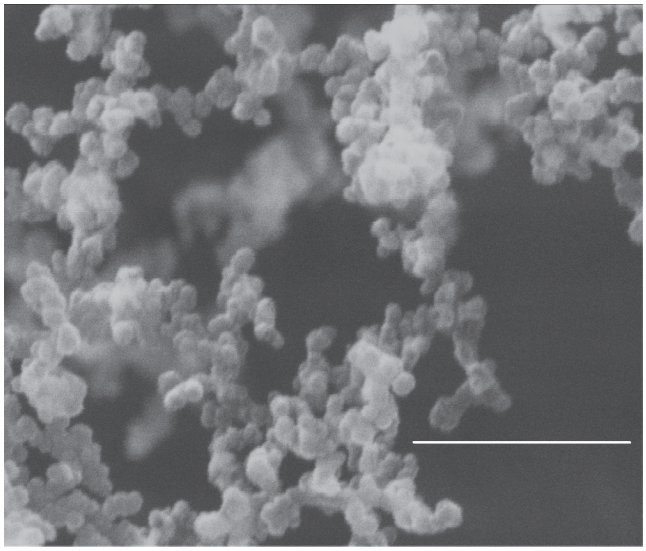

Fig. 2. Examples of ZnO nanoparticles bound by bacteriophages exposing specific peptides on their capsids. A scanning electron micrograph, taken with bacteriophage M13 exposing the ZnO-binding peptide, described by Golec et al. (2012); the bar represents 500 nm. The micrograph was provided by Dr. Kamila Żelechowska (with permission)

crucial under specific conditions (Golec et al., 2012). If this is true, one might speculate that peptides of various sequences might be optimal for different applications. Hence, identification of many variants of ZnO-binding peptides should not be considered an overabundance of slightly different variants of the same tool, but rather they may be recognized as a chance for the employment of alternative tools to achieve different goals in studies into many applications of ZnO nanoparticles.

Examples of ZnO nanoparticles bound by peptides exposed on bacteriophage capsids are shown in Fig. 2. Synthesis of ZnO nanoparticles with the use of chemically obtained peptides (whose sequences have previously been determined by the phage-display technique) may result in the aggregation of these nanoparticles. However, the results shown in Fig. 2 indicate that this is not the case when peptides are still exposed on the surface of bacteriophages, and nanoparticle synthesis occurs on virions. Even if the structures are complex, they are composed of separate nanoparticles of ZnO (Fig. 2).

Concluding remarks

The employment of peptides specifically binding ZnO, and selected with the use of the phage display method, appears to be an encouraging alternative for the synthesis of nanoparticles. This approach may have a high level of impact on bionanotechnological applications, as zinc oxide nanomaterials are widely used in biomedicine. Further development of novel biosensors, diagnostic tools, and therapies based on such materials is within reach. It is interesting that papers describing the identification of novel ZnO-binding peptides are relatively rare in the literature, while – at the same time – the synthesis of ZnO nanoparticles at room temperature and in the absence of harsh chemical procedures is still far from satisfactory. ZnO-binding peptides provide the basis for developing an inexpensive and eco-friendly method for such synthesis which can also be free from chemical contaminations. However, further research on the optimization of both peptide sequences and reaction conditions is necessary to achieve this goal. This may also be true for nanoparticles of other materials, particularly metal oxides.

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