DIFFERENT TYPES OF PROTEIN NANOSTRUCTURES BASED ON COORDINATIVE BOND

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Introduction

Recent advances in the field of proteomics have allowed us to better understand the role and function of several peptides and proteins. As a result many new peptide or protein-based drugs are being discovered every day and offer new ways to treat diseases. Proteins play an increasingly important role as therapeutic agents. The use of these potentially beneficial compounds as drugs for cancer and other diseases may, however, be severely limited by their poor permeability through biological membranes, fragile structure of the biomolecules and the body's ability to rapidly remove them from the blood stream. At present, protein drugs are usually administered by parenteral route, which is connected to inconvenient and painful injections as well as fluctuations of blood drug concentration. Nanotechnology approaches focus on formulating therapeutic agents in biocompatible nanostructures such as nanoparticles, nanocapsules, micellar systems, and conjugates. These nanometre sized delivery systems can improve the timed release of drug molecules, provide targeted delivery, improve poor bioavailability, and improve the stability against enzymatic degradation.

One of our recent research challenges has been exploitation of the active principle of protein aggregation, involving metal coordination of specifically designed protein analogues. Protein aggregation on the surface of solid particles can be realized in two ways: either by pre-decorating the surface of inorganic nanoparticles with a transition metal [1] or using polyfunctional chelating agents to self-assemble protein molecules. The crucial property of metal coordinative binding is its reversibility, enabling controlled release of individual protein molecules upon suitably changed environmental conditions, such as pH decrease or presence/supply of other chelating compound(s). This principle is widely exploited in immobilized metal affinity chromatography [2].

Specifically designed analogues of tumour necrosis factor alpha (TNF-α) with surface exposed clusters of histidines (LK801) [3] and histidine tags attached to N-terminus (His10-TNF and H7dN6TNF) [4] served as model proteins for protein nanostructures formation.

Experimental

Zinc-phosphate nanoparticles:

Zinc-phosphate nanoparticles were produced by the so-called wet precipitation method, where the reaction occurs in a solution. Water solutions of 1M $ZnCl_2$, 0.2M H_3PO_4 and 1M Na_2CO_3 were used as starting reagents. Particle formation was achieved in 1 hour at RT and pH around 7.0. Later 3 different TNF-α analogues were added and bound to the phosphate particles. A part of the solution was dried for SEM analysis.

The amount of TNF-α analogues bound to the particles was analysed with SDS-PAGE. Prior to that, particles were centrifuged and bound TNF-α was released after addition of a phosphate buffer with low pH (around 5,0) or, alternatively, by a phosphate buffer containing 0,5M concentration of imidazole. In the same way the release profile of proteins from particles in close to physiological conditions was measured.

Biological activity of released TNF-α analogues was determined as specific cytotoxicity on mouse fibroblast L-929 cell line.

Self-assembled nanoparticles:

Self-assembled nanostructures were prepared with different chelating agents (phytic acid and 1,4,8,11-Tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA)). First 50 µl of 50 mM chelating agent was added to PBS buffer and pH was adjusted to 7.0. Later TNF-α analogue (0,5 mg) was added. pH was controlled at 7.0 and 0,3 M $Zn(SO)_4$ was slowly added while stirring. The total volume of mixture was around 4 ml.

The size of the nanostructures was measured with dynamic light scattering (DLS) and later analysed also with size exclusion chromatography (SEC) and electron microscopy.

Results and discussion

Zinc-phosphate nanoparticles had a spherical shape and the size around 50 nm as confirmed with SEM (Fig. 1) and DLS. TNF-α analogues were successfully bound onto the particles. Release was achieved with lowering the pH or with the addition of imidazole or strong chelating agent - EDTA, which proved the coordinative nature of the bond. Released TNF-α analogues retained their biological activity and the binding was reversible.

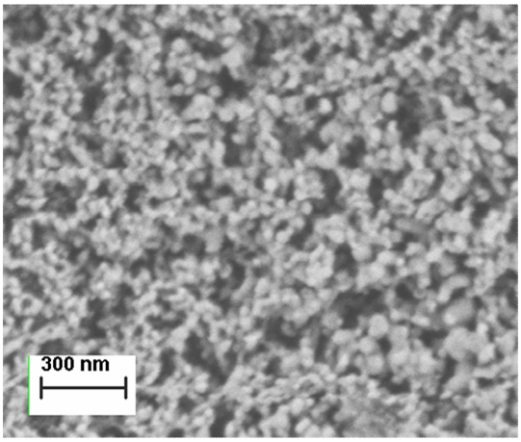

Fig. 1: SEM image of zinc phosphate nanoparticles containing His10TNF.

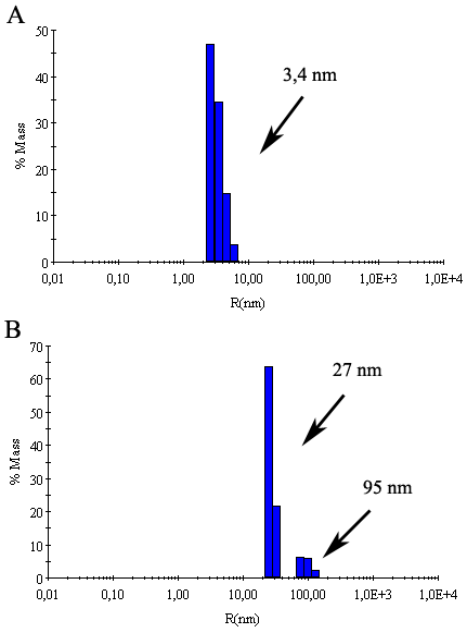

Fig. 2: Size distribution profile (measured with DLS) of H7dN6TNF protein alone (A) and in the form of self-assembled nanoparticles using chelating agent TETA and zinc ions (B).

Protein self-assembly nanostructures were formed using different polyfunctional biocompatible chelating agents in combination with 3 histidine rich TNF-α analogues and zinc ions. The controlled formation of self-assembly nanostructures was achieved. The key in controlling the size of the aggregates is the type and amount of the chelating compound and amount of zinc ions added. The formation of aggregates was followed by SEC, SEM and DLS. The radius of H7dN6TNF alone was 3,4 nm (Fig. 2A) and the radius of self-assembled H7dN6TNF nanoparticles using chelating agent TETA was mostly 27 nm (Fig. 2B) as measured with DLS.

Conclusions

Histidine-rich TNF-α analogues were successfully bound onto zinc-phosphate nanoparticles or self-assembled into protein nanoparticles with the use of different chelating agents and zinc ions.
The coordinative nature and consequent reversibility of binding was proved and, most importantly, the released TNF-α analogues retained their biological activity.
In the case of nanoparticles containing TNF-α analogues with reduced biological activity (H7dN6TNF and His10-TNF) an increased immune response is expected after administration to the testing animals. Enhanced formation of antibodies against TNF-α would be advantageous serving as a basis for developing new drugs for chronic diseases associated with pathogenically elevated levels of TNF-α (rheumatoid arthritis, Crohn's disease, psoriasis, etc.).
On the other hand, nanoparticles containing TNF-α analogue LK801 could be used in anti-cancer therapy, where slow release of individual, active molecules from nanoparticles would presumably result in sustained anti-tumor activity with reduced side effects.

References

1. Bele, M. et al. Zinc-decorated silica-coated magnetic nanoparticles for protein binding and controlled release. *J. Chromatogr. B.*, **867**(1) (2008) 160-4.
2. Gaberc-Porekar, V. et al. Perspectives of immobilized-metal affinity chromatography. *J. Biochem. Biophys. Met.*, **49** (2001) 335-60.
3. Novaković, S. et al. New TNF-α analogues: a powerful but less toxic biological tool against tumours. *Cytokine.*, **9** (8) (1997) 597-604.
4. Fonda, I. et al. Attachment of histidine tags to recombinant tumor necrosis factor-alpha drastically changes its properties. *Scientific World Journal.*, **2** (2002) 1312-25.