Enzyme-mediated disintegration of polyelectrolyte microcapsules loaded with DNA and proteins

T. Borodina[1], L. Rumsh[2], S. Kunizhev[1], G. Sukhorukov[3], O. Kreft[4], E. Markvicheva[2*]

[1] Stavropol State University, Stavropol, Russia; tatiana_borodina@hotmail.com
[2] Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia [3] Queen Mary University of London, , London, UK [4] Max-Planck-Institute of Colloids and Interfaces, Potsdam-Golm, Germany

Introduction

In recent years, the development of new drug carriers for pharmaceutically active substances have attractive much attention, in order to construct controlled and sustained release drug delivery systems (DDS). Several systems, such as micro- and nanoparticles are used as DDS because of their specific properties and applications. For instance, polymeric microparticles can offer many advantages, such as controlled drug release, site-specific drug delivery, and protection of sensitive drugs. Polymeric microcapsules can be used as carriers for delivering proteins, enzymes or DNA to a known position in the human body while preventing them from unwanted decomposition. In this case a promising type for DDS is represented by hollow polyelectrolyte capsules. Their fabrication is based on the layer-by-layer (LbL) self-assembly of oppositely charged polyelectrolyte onto a surface of colloidal microparticles. Drug release from hollow capsules is an important task in many fields of biomedical research. Hollow microcapsules have great deal of attention as novel DDS since their membrane permeability can be controlled. Remote opening systems based on ultra-sound, magnetic fields or IR-Laser have been recently demonstrated as well as the release via stimuli like pH, temperature or ionic strength. We propose a sustained release system in which the encapsulated DNA or proteins can be released from hollow capsules by the enzymatic degradation of the capsule membrane within a range of several minutes, hours or days. For many applications, enzymatic degradation is advantageous because of the ubiquitous occurring enzyme in the body. Two approaches to release protein/DNA from the polyelectrolyte microcapsules can be proposed. The enzyme providing decomposing hollow polymeric microcapsule can be encapsulated within microcapsule core or can occur outside of the microcapsule, namely in the environment. First approach deals with formation of microcapsules on an enzyme-containing template. In the case of this system, core dissolution leads to the enzyme release into the capsule interior promoting continuous digesting the surrounding polymeric shell. The second approach is based on the degradation of microcapsules following on the outside treatment with the enzyme. Therefore both approaches can provide drug release from the microcapsules. The aim of the current research was to fabricate biodegradable microcapsules loaded with protein or/and DNA, and to demonstrate their release mediated by enzyme which can digest microcapsule shell.

Materials and methods

Poly-L-arginine (P-Arg, MW 15 000 – 70 000), poly-L-aspartic acid (P-Asp, MW 15 000), poly-L-lysine (PLL, MW 15000 30000), sodium alginate (Alg), medium viscosity, fluorescein isothiocyanate (FITC), ethylenediaminetetraacetic acid (EDTA), DNA (Type IV: from herring testes), N-Benzoyl-L-tyrosine ethyl ester (□□□□), $CaCl_2$ and Na_2CO_3 were purchased from Sigma-Aldrich (Munich, Germany). Pronase® was obtained from Roche (Mannheim, Germany). Chymotrypsin (Chym, EC 3.4.21.1) and Trypsin (Tryp, EC 3.4.21.4) were purchased from Fluka. $CaCO_3$ microparticles (3-5 μm) were obtained as described earlier by Volodkin et al. (Volodkin et al., 2004). All compounds were used without further purification. The water used in all experiments was prepared using a three stage Millipore Milli-Q Plus 185 purification system.

Preparation of Type I (P-Asp/P-Arg)$_{3.5}$ microapsules. Pronase- and DNA-loaded $CaCO_3$ particles have been prepared according to the coprecipitation-method (Volodkin et al, 2004). Briefly, 1.5 ml H_20, 0.615 ml 1M $CaCl_2$, 0.615 ml 1M Na_2CO_3, 500 μl Pronase solution (1, 3 or 5 mg/ml) and 500 μl dsDNA solution (5 mg/ml) were rapidly mixed and thoroughly agitated on a magnetic stirrer for 20 seconds at RT. For control, we also prepared particles containing DNA without Pronase. Polyelectrolyte multilayer assembly was fabricated on $CaCO_3$ particles by alternative adsorption of 7 monolayers of P-Asp and P-Arg from aqueous solutions (2 mg/mL; 0.15 M NaCl) according to the LbL technique (Donath et al, 1998). $CaCO_3$ cores were finally removed by complexation of Ca^{2+}-ions with an ice-cold EDTA-solution (0.2 M; pH 7.5). Resulting Pronase- and DNA-filled, or control capsules were kept on ice before being subjected to degradation- and release-studies.

Preparation of Type II (PLL/Alg)$_3$ Capsules. Chymotrypsin-loaded microcapsules were prepared by adsorption-method (Volodkin et al, 2004). Briefly, porous $CaCO_3$ particles were exposed to protein solutions (Chym, 5 mg/ml) for 2 h. Then after washing with distilled water microparticles with adsorbed protein were used for fabrication of the polyelectrolyte microcapsules via LbL self-assembly of oppositely charged Alg and PLL (2 mg/mL; 0.15 M NaCl). The $CaCO_3$ core was dissolved by the EDTA treatment as described above. Finally Alg/PLL microparticles containing 6 layers of polyelectrolyte were prepared.

Determination of encapsulation efficiencies. Pronase content was tested before and after coprecipitation-step by using the Coomassie brilliant blue G 250 staining protocol according to the literature route (Bradford, 1976). Obtained differences to given initial amounts of Pronase were regarded as the fraction incorporated into particles. The DNA and Chym concentrations in capsules were determined in a similar way by measuring the OD_{260} and OD_{280} of the supernatants by UV/Vis-spectroscopy.

Enzymatic degradation and DNA release from Type I microcapsules. DNA release was determined after removing $CaCO_3$ cores as described earlier (Borodina et al., 2007). Briefly, the core was dissolved by Ca^{2+}-ions with an ice-cold EDTA-solution (0.2 M; pH 7.5) and washing 3 times with ice-cold water. Degradation process of the microcapsules was started by warming up the samples to RT. At defined time intervals 3 samples were centrifuged and the supernatants were tested for DNA contents. The calculated initial DNA content per capsule was set as 100 %.

Enzymatic degradation and protein release from Type II microcapsules. Aliquots of Chym-loading hollow microcapsules (50 μl) were added to aliquots (100 μl) of Tryp solution. After a predetermination time interval, the suspension was fractioned and centrifuged. The amount of protein released was determinate by measuring the OD_{280} of the supernatant.

Characterization of microcapsules. Morphology of microparticles and hollow microcapsules was studied by Confocal Laser Scanning Microscopy (CLSM, Leica, Germany), Scanning Force Microscopy (Digital Instruments Inc., Santa Barbara, CA), Scanning Electron Microscopy (SEM, Gemini Leo instrument). Electrophoretic mobilities were measured using the Malvern Zetasizer 3000HS Instrument at RT.

Results and Discussion

Preparation of protein- and DNA-loaded microcapsules. For the preparation of polyelectrolyte microcapsules we used porous $CaCO_3$ microparticles as templates. Preparation of this particles has been a subject of many studies due to its great important properties, such as porous structure with high surface area, spherical shape, biocompatibility, biodegradation capability. Figure 1 displays SEM photos of different magnifications for $CaCO_3$ microparticles preparing by mixing two salt solutions at appropriate conditions.

Figure 2 shows a principal fabrication scheme. The loading of $CaCO_3$-templates with Chym was achieved by the physical enzyme adsorption on the surface of $CaCO_3$ core (step A). The microparticles containing Pronase and DNA were prepared by the co-precipitation method (step B).

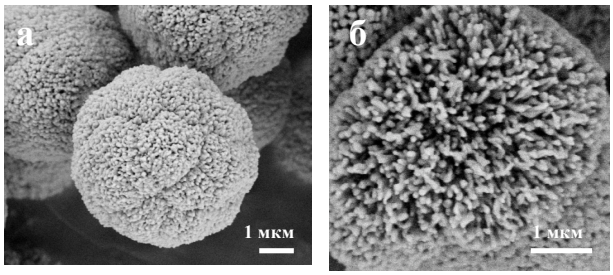

Fig. 1. SEM images of $CaCO_3$ microparticles

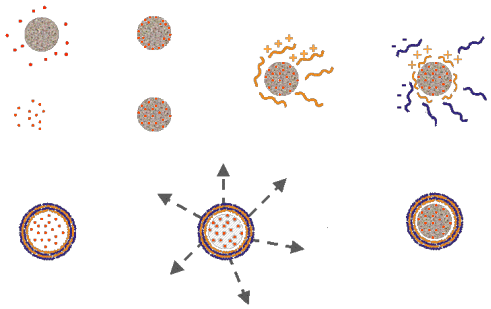

Fig. 2. A scheme for capsule fabrication and encapsulation of proteins/DNA into capsules.

These particles were employed as template core for the electrostatic layer-by-layer assembly of oppositely charge polyelectrolyte (steps C, D, E). At the final step, the core-shell particles were converted into hollow microcapsules by calcium ions complexation with EDTA (step F) resulting in a complete withdrawal of all calcium carbonate constituents from the template. Consequently, the $(P\text{-}Asp/P\text{-}Arg)_{3,5}$ and $(Arg/PLL)_3$ hollow microcapsules were prepared (step G).

DNA release from $(P\text{-}Asp/P\text{-}Arg)_{3,5}$ microcapsules induced by encapsulated Pronase activity. CLSM observations of $(P\text{-}Asp/P\text{-}Arg)_{3,5}$ microcapsules loaded with Pronase were showed that after the core dissolution the majority of microcapsules was dissolved (data not shown). The microcapsules without Pronase (control) did not degrade after the decomposition of the $CaCO_3$ template.

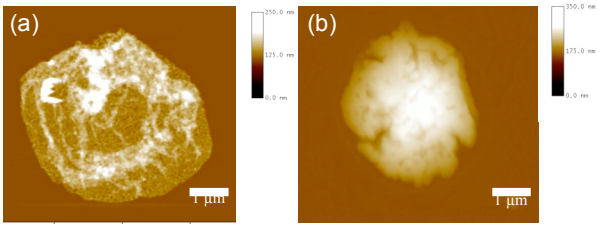

Fig. 3. AFM images of the degradation of $(PAsp/PArg)_{3.5}$ microcapsules due to enzymatic activity of encapsulated Pronase.

To investigate the morphology of microcapsules during the Pronase-driven decomposition of the shell AFM was used (Fig.3). Capsules shrinking from 5 ± 1 μm to a final diameter of 2.5 ± 0.2 μm as an immediate response to the core-decomposition was observed. Figure 3a illustrates the control sample without Pronase. These results indicate that a microcapsule shape was changed as a result of its destruction at biodegradation.

By exploiting the biodegradable property of the microcapsules, it could be possible to control the release of encapsulated substances from microcapsules by controlling the amount of encapsulated Pronase. We investigated a few types of microcapsules containing DNA and various Pronase concentrations in the interior. The effect of the Pronase amount on the encapsulated DNA release is shown in Figure 4. One can see that at Pronase concentration 12.3 mg/mL in microcapsules 90% of the DNA was released during the first 2,5 hours. For the microcapsules in which the initial enzyme concentration was 4.6 mg/mL) DNA accumulation (more than 90 %) in the supernatant was found in 30 hours. In the case of higher Pronase amount (20 mg/mL), microcapsules were completely degraded immediately after the core dissolution.

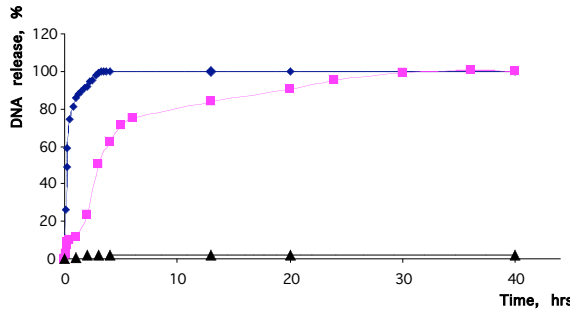

Fig. 4. DNA release from microcapsules containing 12.3 mg/mL (♦) and 4.6 mg/mL (■) of Pronase. The control microcapsules were without Pronase (▲).

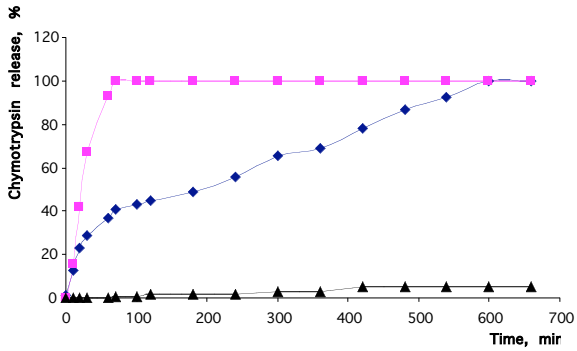

Fig. 5. Chymotrypsin release from microcapsules induced by treatment with trypsin at various concentrations : 10^{-4} M (■) and 10^{-6} M (♦). The control microcapsules were without Tryp (▲).

Chymotrypsin release from $(Arg/PLL)_3$ microcapsule capsules induced by trypsin treatment. Since PLL can be digested by proteolytic enzymes, the enzymatic degradation of $(Arg/PLL)_3$ microcapsules using Tryp treatment was studied. Optical observations of the hollow microcapsules after their incubation in a Trypsin solution (10^{-4}M) were carried out. After the enzyme treatment for 1 hour the microcapsules were dissolved. On the other hand, the microcapsules immersed in the same conditions without Tryp (control) were not destroyed. The influence of Tryp concentration on Chym release profiles by varying the amount of Tryp was investigated. Figure 4 shows that the rate of protein release depended on the Tryp concentration. In the presence of 10^{-4}M Trypsin, more than 90 % of the encapsulated Chym was released in 70 min. In the case of a smaller Tryp concentration (10^{-6}M), the total release time was around 600 min.

Conclusions

We proposed novel sustained release systems for protein or/and DNA encapsulation which are based on biodegradable polyelectrolyte microcapsules. The release of encapsulated protein/DNA can be provided by enzyme-mediated degradation of the microcapsule shell. The protein/DNA release can be controlled by varying enzyme concentration.

Acknowledgment

Authors thank DAAD and FEBS for the support of Tatiana Borodina.

Reference

D. Volodkin et al. (2004) *Matrix polyelectrolyte microcapsules: new system for macromolecule encapsulation.* Langmuir, 20, 3398-3406.
D. Volodkin et al. (2004) *Protein encapsulation via porous CaCO microparticles templating.* Biomacromolecules. 5(5), 1962- 1972.
Donath et al. (1998) *Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes.* Angew. Chem.-Int. Edit. 37, 2202-2205.
M.M. Bradford (1976) *Analytical biochemistry*. 72, 248.
T. Borodina (2007) *Controlled Release of DNA from Self-Degrading Microcapsules*. Macromol. Rapid Com. In press