P-061

Enzyme-catalyzed disassembly of multicompartment particles and capsules with polyelectrolyte shell formed of polypeptides

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INTRODUCTION AND OBJECTIVES

Polyelectrolyte microcapsules formed by stepwise adsorption of oppositely charged polyelectrolytes onto the surface of colloidal particles can be used as microcontainers, microreactors and sensors (De Geest 2009). Multicompartment capsules proposed in (Delcea 2010) which are several capsules attached to each other, are promising objects for simultaneous delivery of several compounds and as intracellular sensors, when capsules with sensors to different substances are combined in one carrier. It is important to study detaching of parts of multicompartment capsule. To perform such detaching we proposed to decompose the biodegradable shell by enzyme action.

Multicompartment particles were formed on the base of silica microparticles covered by polyelectrolyte shell including biodegradable polypeptides Poly(L-arginine) and Poly(L-aspartic acid). The polyelectrolyte shell can be destroyed by Pronase which is a mix of proteases breaking down virtually all proteins and synthetic polypeptides into individual amino acids (Borodina 2009). Such mechanism of detaching can be realized inside cells, where polypeptide shell, as was shown in (De Geest 2006) can be destroyed by enzymes containing in the cell.

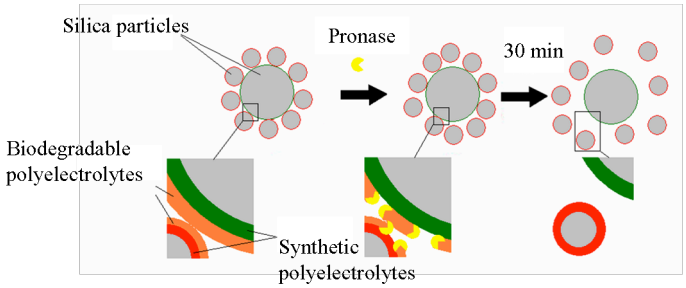

Figure 1 : Scheme of structure and disassembly of multicompartment particles

MATERIALS AND METHODS

Silica microparticles with sizes 4.80±0.19 μm and 0.58±0.02 μm were purchased from microparticles GmbH. Poly(allylamine hydrochloride) (PAH, Mw 70 kDa), poly(sodium 4-styrenesulfonate) (PSS, Mw 70 kDa), poly(L-arginine) (pArg, Mw 70 kDa), poly(L-aspartic acid) (pAsp, Mw 15 kDa), poly(L-glutamic acid) (pGlu, Mw 50-100 kDa), tetramethylrhodamine isothiocyanate-dextran (TRITC-dextran, 70 kDa), fluorescein isothiocyanate-dextran (FITC-dextran, 70 kDa) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich. Pronase was purchased from Roche.

Confocal laser scanning microscopy (CLSM) images were recorded with a Leica TCS SP confocal scanning microscope (Leica, Germany) in inverted microscope mode equipped with a 100×/1.4-0.7-oil immersion objective.

RESULTS AND DISCUSSION

Silica microparticles with sizes 4.80 μm and 0.58 μm coated with polyelectrolyte shell were used as inner and outer subcompartments for fabrication of multicompartment particles respectively. Both synthetic (PAH and PSS) and biodegradable (pArg and pAsp) polyelectrolytes were adsorbed on the particles by layer-by layer (LbL) deposition technique (Sukhorukov 1998). For visualization one layer of TRITC-dextran was incorporated in the shell of outer subcompartments and one layer of FITC-PAH was incorporated in the shell of the inner ones. Inner part of the shell was formed of synthetic polyelectrolytes and the outer part comprised biodegradable polypeptides. The polyelectrolyte shell of the outer subcompartments had structure PAH/PSS/PAH/TRITC-dextran/pArg/pAsp and inner subcompartments – $(PAH/PSS)_2$/FITC-PAH/PSS/pArg/ pAsp/pArg. The outer layers of larger and smaller particles had an opposite charge, so the small particles were attached to the surface of the larger ones due to electrostatic interaction of their outer layers. To adsorb outer subcompartments onto the surface of the inner ones equal volumes of their suspension with concentration 7.5 mg/mL and 1.5 mg/mL respectively were mixed and shaked for 10 minutes. Obtained multicompartment particles are shown on Fig. 2a.

To perform detaching of the particles they were subjected to action of Pronase. The outer synthetic layers of both inner and outer subcompartments were negatively charged, so we supposed that after degradation of polypeptide part of the shell the outer subcompartments will be able to detach from the surface of the inner ones. The multicompartment particles were incubated in 20 mg/ml Pronase solution at 37 ºC for one hour. Complete detaching of the outer particles from the surface of the inner ones was observed after 30 minutes of incubation in enzyme solution (Fig. 2b).

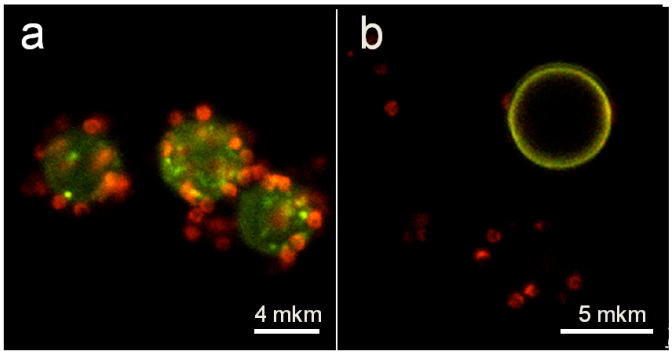

Figure 2 : CLSM images of multicompartment capsules (a) before and (b) after enzyme action.

For investigation of enzyme degradation of biodegradable polyelectrolyte shell capsules consisting of polypeptides were formed. Dependence of integrity of capsules shell on number of polyelectrolyte layers and enzyme concentration was studied. Capsules consisting of 4 and 8 bilayes of pArg/pGlu were formed. $CaCO_3$ cores were fabricated by method, described in (Antipov 2003). For visualization FITC-dextran was encapsulated. For it before adsorption of polyelectrolytes $CaCO_3$ cores were mixed with FITC-dextran (1 mg/mL) for 30 min at constant shaking. PArg and pGlu were adsorbed on particles surface using the LbL deposition technique from solutions with concentration 2mg/mL in 0.15 M NaCl. To obtain hollow capsules the cores were dissolved by EDTA.

To study degradation of the capsules they were incubated in 1 or 5 mg/ml Pronase solution at 37 ºC for 2 hours. CLSM images show that degradation of the shell takes place. It was observed that capsules comprised eight bilayers are more stable than four bilayers capsules and decrease their size more slowly (Figure 3): after 1 hour of enzyme action the average size of capsules decreases from initial 5 µm to 1 µm for four bilayer capsules and to 3 µm for eight bilayer capsules.

Increase of concentration of Pronase solution up to 5 mg/mL lead to faster capsule degradation: after 30 min of enzyme action the average size of four bilayers capsules decreases from initial 5 µm to 4 µm for concentration 1 mg/mL and to 1 µm for concentration 5 mg/mL.

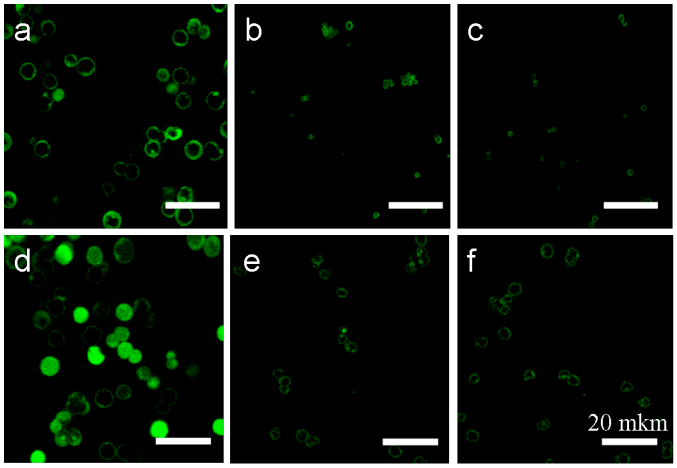

Figure 3 : (a-c) CLSM images of 4 bilayer capsules captured (a) before adding of enzyme solution and (b) after 60 min and (c) after 90 min of enzyme reaction;
(d-f) CLSM images of 8 bilayer capsules captured (d) before adding of enzyme solution and (e) after 60 min and (f) after 90 min of enzyme reaction (Pronase concentration 1 mg/mL).

CONCLUSIONS

Multicompartment particles were formed using layer-by layer technique and disassembled by enzyme reaction. Enzyme degradation of the capsules formed of biodegradable polyelectrolytes in different conditions was studied. It was shown that enzyme degradation of the shell depends on the number of polyelectrolyte layers and on enzyme concentration.

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ACKNOWLEDGEMENT

I. Marchenko thanks FEBS scholarship for support.