Amino Acid-Based Redox-Responsive Nanocapsules for Intracellular Protein Delivery

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Introduction: Protein-based therapeutics has gained a lot of interest as it possesses unique advantages over conventional gene therapy approaches. More specifically, intracellular delivery of a functional protein does not alter the genetic makeup of the cell, a primary cause for tumorigenicity, and is therefore a safer therapeutic alternative. However, cytosolic protein delivery is hampered by limitations such as low serum stability, nanocarrier structural properties, and poor endosomal escaping ability of the protein cargo upon cellular internalization. Here we report the use of amino acid derived, redox-responsive protein nanocapsules (NCs) for effective delivery of a protein by exploiting the reducing environment of the cytosol upon cellular entry.

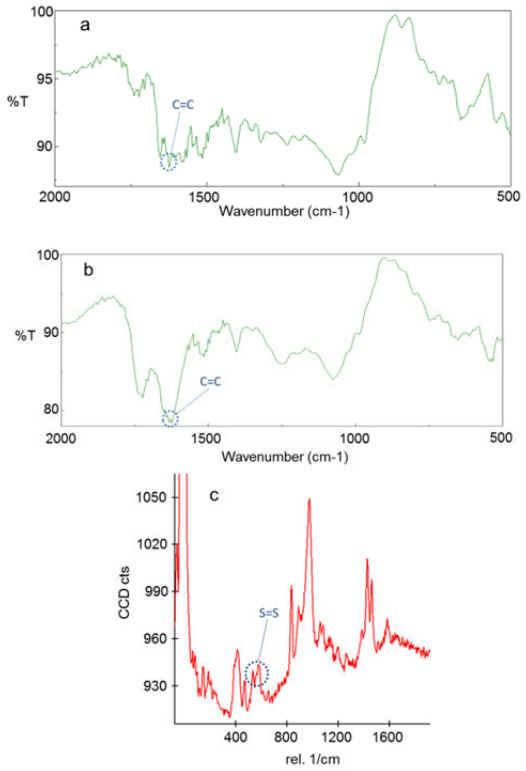
Methods: Amino acid-based monomers, acryloyl lysine (Ac-Ly) and acryloyl ornithine (Ac-Or), and disulfide crosslinker, N,N'-bis(acryloyl)cystamine (BAC), were synthesized using previously published protocols.[1,2] Protein NCs were prepared as described previously.[3,4] Briefly, Ac-Or (1 mg/ml, PBS) was added to a bovine serum albumin solution (BSA, 250 ug in 500 uL of PBS) and was left to stir for 10 min at 4 °C following which Ac-Ly (1 mg/ml, PBS) was added. Different crosslinkers, diethylene glycol dimethacrylate (DEGDMA) for non-responsive NCs and N,N'-bis(acryloyl)cystamine (BAC) for disulfide-crosslinked NCs, was then added 5 min after addition of Ac-Ly. Radical polymerization was initiated by adding ammonium persulfate (0.5M, 50 uL, PBS) and ascorbic acid (0.5M, 50 uL, PBS) and the reaction was allowed to proceed for 90-120 min. Finally, unreacted monomers, crosslinker and initiators were removed by dialysis in 10 mM pH 7.4 PBS.

The protein nanocapsules were analyzed by Fourier transform infrared (FTIR), transmission electron microscopy (TEM) and dynamic light scattering (DLS) methods. Protein release from the protein NCs was evaluated using SDS-PAGE, intracellular protein delivery through fluorescence microscopy and cytotoxicity using MTT assay.

Results: The FTIR spectra of the synthesized amino acid-based monomers confirmed the presence of characteristic C=C band at 1635 cm^{-1} (Figure 1a & b). The presence of the S=S band at 550 cm^{-1} for the synthesized BAC crosslinker was confirmed by Raman technique (Figure 1c). The zeta potential and the size of the amino acid-based monomers and native BSA are given below.

	Particle Size (nm)	**Zeta potential** (pH 7)
Native BSA	6	- 4.2 mV
N-acryloyl ornithine	174	+16 mV
N-acryloyl lysine	186	-30 mV

Figure 1



The particle sizes of the synthesized protein NCs with DEGDMA or BAC were found to be ~300 nm. The size distribution of the protein encapsulated NCs were further confirmed by TEM, in which the NCs were found to possess a spherical morphology. SDS-PAGE results demonstrated that the disulfide crosslinked protein NCs released BSA at a higher rate compared to non-responsive DEGDMA crosslinked NCs in the presence of 10 mM dithiothreitol. Fluorescence microscopy confirmed intracellular delivery of FITC-conjugated BSA in the cytosol of cells. MTT assay demonstrated more than 80% cell viability for the protein NCs.

Conclusions: We have developed a new class of protein NCs that can reversibly release an encapsulated protein in a reducing environment of the cytosol of the cell. This simple yet powerful strategy can be exploited for intracellular delivery of protein macromolecules for various tissue regenerative and therapeutic applications.

References : [1] Romanski et al. *J. Polym. Sci., Part A: Polym. Chem.* 2012;50:542-560; [2] Han et al. *J. Polym. Sci., Part A: Polym. Chem.* 2009;47:4074–4082; [3] Zhoa et al. *Biomaterials* 2011;32:5223-5230. [4] Wen et al. *Adv. Mater.* 2011; 23:4549-4553.

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