Amphiphilic polysaccharide nanoballs with chaperon-like function for protein delivery

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ABSTRACT

Amphiphilic enzymatically synthesized glycogen (ESG) was synthesized as a new platform for protein DDS. The amphiphilic ESGs by modification of cholesterol groups or dodecyl groups (CHESG or C12ESG) showed chaperon-like function for protein refolding. Cationic CHESG strongly interacted with proteins and stabilized them against thermal denaturation. The cationic CHESG effectively internalized proteins into HeLa cells.

INTRODUCTION

We developed self-assembled nanogels by amphiphilic polysaccharides as an effective nanocarrier for drug delivery system. Cholesterol-bearing pullulan (CHP), where small number of cholesterol groups are linked to the linear polysaccharide pullulan, forms stable nanogel by self-assembly in water. CHP nanogels can complex soluble proteins and stabilize the proteins against thermal stress by encapsulation. Furthermore, CHPs act as artificial molecular chaperones that assist refolding of denatured proteins to their native forms during the release process. We report here enzymatically synthesized glycogen (ESG) as a new template polysaccharide. ESG is a highly branched (1→4)(1→6)-linked α-glucan and monodispersed spherical nanoparticle (molecular weight: 2.2 × 10⁶; hydrodynamic radius ($R_H$): 13.5 nm; number-average chain length: 9.3; approximately 1400 non-reducing ends) in the form of a polysaccharide nanoball [3]. We synthesized amphiphilic ESGs with hydrophobic groups such as cholesterol groups (CHESG) or alkyl groups (C12ESG) and also cationic amphiphilic ESG introduced diethyl-ethylenediamine group (CHESG-DEAE). Chaperon-like activity for refolding of chemically denatured protein was investigated by the amphiphilic nanoballs (Fig. 1) [4]. The intracellular protein delivery into HeLa cells was estimated using confocal laser scanning fluorescence microscopy (CLSF) [2].

EXPERIMENTAL METHODS

CHESG was synthesized and reported previously[1]. A series of C12ESGs molecules with different degrees of substitution of the dodecyl groups were prepared by reacting the hydroxy groups of pullulan with dodecyl isocyanate in anhydrous dimethylsulfoxide in the presence of dibutyltin dilaurate as a catalyst. C12ESGs of 120, 280 and 380 C12 groups per one nanoball were synthesized. Cationic CHESG (CHESG-DEAE) were synthesized by modification of N,N-diethylethylenediammine (DEAE) to CHESG.
The refolding activity of C12ESG was evaluated in terms of refolding of carbonic anhydrase (CA) as a model enzyme. CA was unfolded and was completely inactivated in 6.0 M guanidine monohydrochloride. After denaturation for 16 h at 25 °C, the refolding of CA was induced by rapid dilution in renaturation buffer in the absence and presence of the nano-balls.

A 1.0 mL of fresh MEM containing the CHESG–DEAE–BSA complex was added to human cervical cancer (HeLa) cells. After co-incubation for 4 h or 24 h at 37 °C in 5% CO2, the cells were washed three times with a fresh medium and were observed by CLSFM.

RESULTS AND DISCUSSION
Hydrodynamic radii \( (R_H) \) of C12ESG nanoballs were approximately 15 nm determined by dynamic light scattering. In the absence of the nanoballs, denatured CA was irreversibly aggregated after rapid dilution. The spontaneous refolding was only 17% of the original enzymatic activity. In the presence of the C12ESG under the same dilution conditions, however, no obvious precipitation was observed following dilution because of inhibition of the irreversible aggregation. The recovered CA activity after 24 h increased with increasing degree of substitution of the dodecyl groups (Fig. 2). These results indicate that CHESG caught the denatured proteins and continuously released the proteins in their folded, active form during the dilution.

We next investigated the protein delivery of the ESG derivatives into HeLa cells. The CHESG-DEAE formed strong and stable complexes with the proteins through hydrophobic and electrostatic interactions. The ESG derivatives–BSA complexes were co-incubated with HeLa cells. The cellular uptake efficiencies of FITC-BSA were significantly increased by using cationic CHESG-DEAE. CLSFM experiments showed that FITC-BSA was effectively delivered by CHESG-DEAE and internalized inside the cells by endocytosis.

CONCLUSION
The C12ESG prevented irreversible aggregation of CA and exhibited chaperone-like activity which is important in protein engineering or protein delivery system. The cationic CHESG was capable of cellular transport of proteins effectively, internalizing a number of proteins into all cells and delivering a portion of the protein to the cytoplasm. The cationic ESG nanoball proved to be useful as a new effective candidate for protein delivery systems.

REFERENCES