Amphiphilic Hyauronic Acid Derivatives as a Potential Carrier for Protein Drug

A Reum Kang[1], Maharajan Sivasubramanian[1], Jae Hyung Park[1,2]
[1]Departments of Advanced Polymer and Nanopharmaceutical Sciences, Kyung Hee University, Gyeonggi-do 446-701, Korea
[2]BioImaging Research Center, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea

Introduction

Molecular chaperone facilitate correction of the improperly folded proteins into its functionally active structure. In recent years, it has been found that amphiphilic nanogels exhibit the molecular charperone-like activity for misfolded proteins [1]. The aim of this research was to develop nanogels for sustained delivery of protein drugs, for which the denatured protein was physically encapsulated into the nanogels throughout the hydrophobic interaction. For this purpose, we have synthesized the amphiphilic hyaluronic acid (HA) derivatives which can form self-assembled nanogels in an aqueous environment. The physiochemical characteristics of the HA derivatives were investigated using ^{1}H NMR, dynamic light scattering (DLS), and flourescence spectrophotometer. The carbonic anhydrase (CAB) as a model protein was denatured in the presence of gaunidinium chloride (GdmCl), followed by physical encapsulation into HA nanogels. Thereafter, the *in vitro* release profiles of CAB from nanogels were investigated.

Methods

Materials HA was purchased from Lifecore Biomed. (Chaska, MN). CAB, p-nitrophenyl acetate (pNPA), α-cyclodextrin(α –CD), β-cyclodextrin(β-CD), GdmCl, and all other materials were obtained from Aldrich (St. Louis, MO, USA).

Synthesis Amphiphilic HA derivatives were prepared by chemical conjugation of 5β-cholanic acid to the backbone of HA, as reported previously [4].

Characterization Chemical structure of the HA derivatives were confirmed using a ^{1}H NMR. The particle sizes of nanoparticles were measured using the DLS with a helium ion laser system which was operated at 633nm.

Denaturation and loading of CAB Native CAB was dissolved Tris-sulfate buffer (pH7.5) containing GdmCl. After denaturation for 12h at 25℃, the mixture was mixed with HA derivative solution in Tris-sulfate buffer. After 4h, the solution was dialyzed, followed by lyophilization. The CAB loading amount was determined using a BCA assay. The interactions between CAB and nanoparticles were demonstrated by using a high performance liquid chromatography.

Activity of CAB Biological activity of CAB released from nanoparticles was determined by the pNPA hydrolysis assay using the microplate reader.

In vitro release profile of CAB The CAB-loaded nanoparticles were dispersed in PBS, which was transferred into the dialysis tube. After the tube was immersed into the medium, the CAB released from nanoparticles was obtained at the predetermined time. The amount of CAB was estimated using a BCA assay.

Results

Denatured CAB was readily encapsulated into HA nanoparticles throughout the physical interaction. The loading efficiency of CAB decreased as the feed ratio of CAB to HA derivative increased, as shown in Table 1. The nanoparticles with larger amount of denatured CAB had smaller particle size, suggesting that the denatured CAB enhanced compactness of the hydrophobic core of nanoparticles. When the nanoparticles were immersed into the physiological solution, CAB was released in a sustained manner for at least up to 9 days. It should be emphasized that more than 90% of the released CAB retained their activity. These results indicated that the nanoparticles could release biologically active CAB for a long period of time.

Table.1 Characteristics of CAB-loaded nanoparticles

Samples	Feed ratio (HACA : CAB)	Size (nm)	Loading efficiency (%)	Loading contents (%)
HACA	-	239.8±7.69	-	-
HACA-CAB 5	1 : 0.05	308.8±1.98	92	2.3
HACA-CAB 10	1 : 0.1	310.1±3.37	76	19
HACA-CAB 20	1 : 0.2	341.7±2.45	58.3	11.6
HACA-CAB 30	1 : 0.3	276.6±5.25	48.8	13.1

Conclusions

The model enzyme, CAB, was successfully encapsulated into the HA nanoparticles in the presence of GdmCl. The enzymatic activity of denatured CAB was recovered by HA nanoparticles, indicating that they play a role as the artificial molecular chaperone. Since the HA nanoparticles can efficiently refold and release CAB in the physiological solution, they might have a good potential as the protein delivery system.

References

1.Yuta Nomura et al., Biomacromolecules, 2005; **6** : 447-452,
2.Nobuyuki Morimoto et al., Biomacromolecules, 2005; **6** : 1829-1834,
3.Yuta Nomura et al., FEBS, 2003; **553** : 271-276
4.Ki Young Choi et al., Biomaterial, 2010; **31**, 106-114.