The New Strategy of Formulation of Human Growth Hormone Aggregate within PLGA Microspheres for Sustained Release

김홍기, 박태관
한국과학기술원 생물과학과
tel (042) 869-2661, fax (042) 869-2610

Abstract

For the sustained release formulation of recombinant human growth hormone (rhGH), dissociable rhGH aggregates were microencapsulated within poly(D,L-lactic-co-glycolic acid) [PLGA] microparticles. rhGH aggregates with 2 - 3 m particle diameter were first produced by adding a small volume of aqueous rhGH solution into a partially water miscible organic solvent phase (ethyl acetate) containing PLGA. These rhGH aggregates were then microencapsulated within PLGA polymer phase by extracting ethyl acetate into an aqueous phase pre-saturated with ethyl acetate. The resultant microparticles were 2 - 3 m in diameter similar to the size of rhGH aggregates, suggesting that PLGA polymer was coated around the protein aggregates. Release profiles of rhGH from these microparticles were greatly affected by changing the volume of the incubation medium. The release rhGH species consisted of mostly monomeric form with having a correct conformation. This study reveals that sustained rhGH release could be achieved by microencapsulating reversibly dissociable protein aggregates within biodegradable polymers.

Introduction

Recently, sustained release formulation of various therapeutic proteins has been attempted to reduce the necessity of their multiple injections to the patients. The most widely used method is to microencapsulate the proteins within biodegradable PLGA polymeric microspheres by double emulsion solvent evaporation method based on water-in-oil-in water ($W_1/O/W_2$). This double emulsion solvent evaporation method, however, elicits protein instability problems during the encapsulation process. So, the protein release profiles from PLGA microspheres seem to be unsatisfactory.[1]

It was reported that rhGH encapsulated PLGA microsphere formulation

prepared with a cryogenic spray drying process successfully finished clinical trials.[2] The addition of zinc as a rhGH stabilizer was claimed be a critical formulation factor to form reversible insoluble aggregates of zinc-rhGH complex. It appears that the formation of zinc-induced rhGH dimeric aggregates play a role in stabilizing rhGH during the formulation and subsequently in exhibiting a continuous monomeric rhGH release from microspheres. Thus, rhGH aggregation pathway can be divided two classes: reversibly dissociable and irreversibly undissociable depending on how rhGH is aggregated.

In this study, it is hypothesized that if rhGH could be aggregated in a reversibly dissociable form, then it is possible to encapsulate the rhGH aggregates within PLGA microspheres to achieve a sustained release for a desired period.

Materials and Method

rhGH was obtained from Dong-A Pharmaceutical Co. (Korea). A partially water miscible organic solvent, ethyl acetate, was used to induce the formation of reversible rhGH aggregates. PLGA microparticles containing the rhGH aggregates were tested for the controlled release of rhGH as functions of surfactant concentration in the incubation medium and the incubation medium volume. It was expected that both surfactant concentration and incubation medium volume affect the rhGH dissolution rate of the dissociable rhGH aggregates. The structural conformation of released rhGH species were characterized by size exclusion chromatography and circular dichroism spectroscopy.

Result and Discussion

The effect of two organic solvents, ethyl acetate and dichromethane, on the aggregation behavior of rhGH dissolved in aqueous solution was tested. The former is more water miscible than the latter. Various rhGH amounts dissolved in deionized water were exposed to ethyl acetate or dichloromethane saturated aqueous phase. Table 1 shows that ethyl acetate saturated water phase induced decrease in transmittance value with increasing the rhGH amount, while dichloromethane did not affect the change in transmittance, which indicates that rhGH aggregated and precipitated when using a partially water soluble organic

solvent in accordance with the conventional thought. The size of rhGH aggregate particles is around 2 - 3 m in diameter as determined by a dynamic light scattering method.

Figure 1 shows the effect of dichloromethane and ethyl acetate on the re-solubilization of rhGH as a function of homogenization time during a simulated process of double emulsion solvent evaporation process in which PLGA polymer was not incorporated into the organic phase.

Figure 2 shows rhGH release profiles from the formulation C microspheres as a function of incubation medium volume. Since it was hypothesized that the release rate of rhGH from the microspheres is dependent on thermodynamic equilibrium between reversibly dissociable rhGH aggregates and rhGH monomer, it was expected that change in the incubation medium volume affected the rhGH dissolution rate from the microspheres. Decreased concentration of monomeric rhGH in the incubation medium shifts the equilibrium towards dissociating more rhGH monomer species from rhGH aggregates. It can be seen that more amount of rhGH was released in response to increasing the volume of incubation medium, suggesting that rhGH was sustained released over time by dissolution of rhGH aggregates in a thermodynamically controlled manner. To confirm whether the released rhGH was a monomeric form, the collected samples after 1 hr incubation were subjected to size exclusion chromatography. It was found that all the samples regardless of the incubation medium volume demonstrated a major rhGH monomer peak with over 92 % homogeneity.

Conclusion

A new formulation approach for encapsulation of rhGH within PLGA microparticles was examined, which was based on the use of ethyl acetate in organic phase as well as in the aqueous extracting medium phase. Reversibly dissociable rhGH aggregate particles could be coated with PLGA polymer, and they exhibited more sustained rhGH release profiles. Modulated rhGH release amount in response to the concentration of rhGH in the incubation medium implies that rhGH release from microparticles occurred via a rhGH dissolution controlled mechanism.

Reference

1. Hong Kee Kim, Tae Gwan Park, "Microencapsulation of Human Growth Hormone Within Biodegradable Polyester Microspheres: Protein Aggregatin Stability and Incomplete Release Mechanism"(1999), Biotech. Bioeng., 65, 6, 659-667

2. Mark A.Tracy, "Development and Scale0up of a Microsphere Protein Delivery System"(1998), Biotech. Prog., 14, 108-115

Table 1. Characteristics of rhGH aggregates in deionized water saturated with organic solvents

	Final rhGH concentration (mg/ml)	*Effective Diameter (㎚)*	*Transmittance (%)*
Water saturated with Ethyl Acetate	0.00	ND	100.0
	0.17	ND	99.6
	0.40	2.27	72.4
	0.54	2.79	56.5
	0.74	2.29	46.8
Water saturated with Dichloromethane	0.74	ND	99.9

ND : not detected

Figure 1.

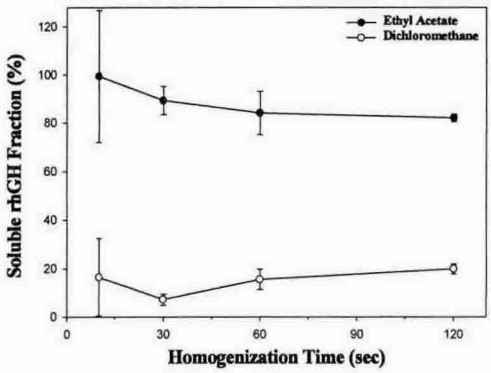
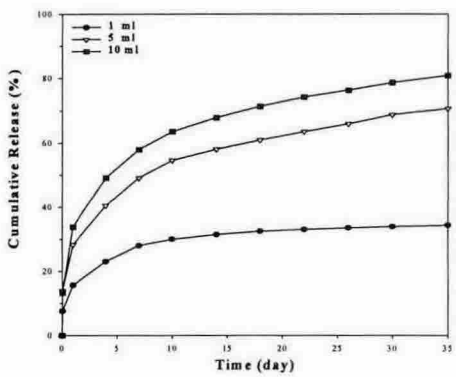
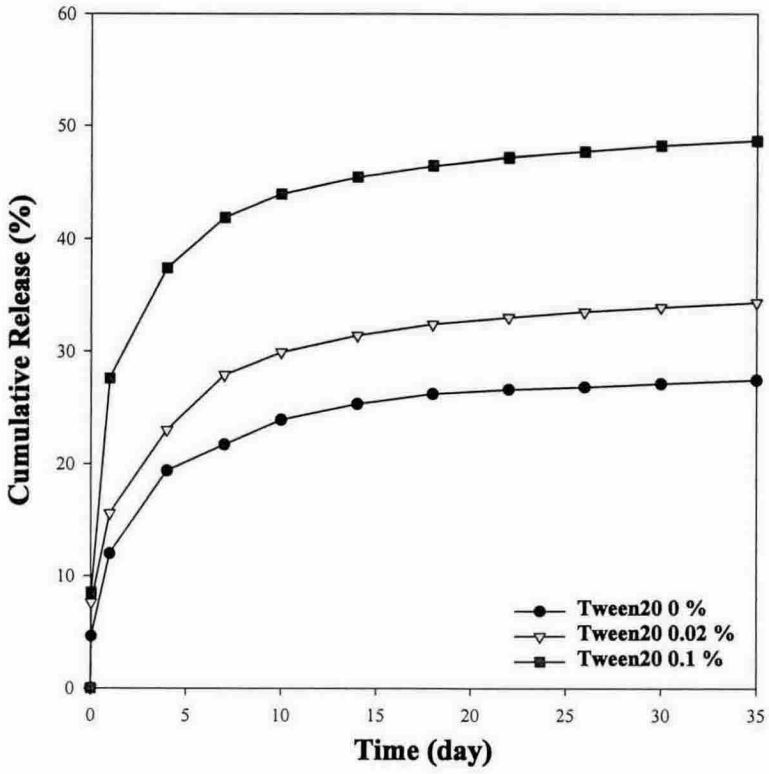


Figure 2.




Cumulative Release (%)
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Time (day)
Tween20 0 %
Tween20 0.02 %
Tween20 0.1 %