Electrostatic and Hydrophobic Dual-binding Interaction Inclusion Nano-complex for Controlled Release of Insulin from Poly(lactide-co-glycolide) Microsphere

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ABSTRACT SUMMARY
For long-term, sustained protein delivery, a new, star-shaped block copolymer was synthesized via the multi-initiation and ring-opening polymerization of aminoacid N-carboxy anhydride on branched oligoethylenimine with a hydrophilic polymer conjugation. The resulting copolymer had strong buffering capacity within the neutral-to-acidic pH range and was complexed with insulin (Ins) via an electrostatic attraction plus hydrophobic interactions, resulting in the formation of a dual-interaction complex (DC) of approximately 30–60 nm in size. This DC tolerated high salt concentrations without destabilization, supporting the existence of hydrophobic interactions, and protected Ins from the organic solvent/water interface. The DC in poly(lactide-co-glycolide) microspheres (PLGA MS) as a long-term Ins delivery formulation was evenly distributed via a double-emulsion method. This formulation possessed near zero-order release kinetics. In streptozotocin-induced diabetic rats, a DC-loaded PLGA MS formulation was able to maintain blood-glucose levels at 200–350 mg/dL for the first two weeks and even lower levels (100–200 mg/dL) for the next two weeks. Thus, a new copolymer and its complex with a drug protein could have potential biological application as a long-term, sustained protein delivery system.

INTRODUCTION
Poly(lactide-co-glycolide) microspheres (PLGA MS) have been intensively investigated as depot systems for long-term protein delivery. However, proteins encapsulated in PLGA MS are often exposed to acidic environments that lead to unwanted hydrolytic PLGA byproducts. As a result, the cargo proteins can become denatured, leading to their reduced bioactivity. To prevent or reduce acid-induced complications, the use of various polymeric additives has been investigated. In one method, a polyelectrolyte complex was formed via ionic interactions between charged proteins and counter-charged polymers.¹ However, the polymer/protein complex dissociated during the fabrication of the PLGA MS and readily released proteins under physiological conditions due to weak ionic interactions. In an effort toward improving polyelectrolytes for long-term protein delivery in PLGA MS, this study designed a new star-shaped copolymer composed of branched oligoethyleneimine. The resulting copolymer was expected to form a dual-interaction complex (DC) with cargo proteins via electrostatic and hydrophobic interactions under physiological conditions (Figure 1).

EXPERIMENTAL METHODS
The complex was prepared by a simple mixing process. Briefly, the Ins solution (HCl 0.01 N, 0.1 mg in 1 mL) was added to a polymer solution (DIW, pH 7.4) with an equivalent volume. The mixture was vigorously vortexed for approximately 10 s and then incubated for 30 min at RT. Complexation conditions of the
polymer/protein complex were studied with the weight ratio (WR) of polymer to protein. PLGA MS was manufactured via the multi-emulsion method.\textsuperscript{3}

RESULTS AND DISCUSSION

\begin{figure}[h!]
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\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Cumulative drug release (wt.%) from PLGA MS in PBS (10 mm and pH 7.4) (n = 3).\textsuperscript{2}}
\end{figure}

In vitro drug (Ins) release studies clearly showed DC effects in the PLGA MS (Figure 2). In general, protein burst release is linked to water channels and pores in the PLGA MS formed during fabrication. Control released 30\% of the loaded Ins as an initial burst, possibly because the hormone was able to pass through the water channels and pores. However, DC loaded MS had a much lower Ins burst release (approximately 15\%), possibly due to the larger size of the DC compared to the water channels and pores in the PLGA MS. After the initial burst, DC loaded MS released an additional 17\% of its Ins load over the next three weeks with a daily Ins release of approximately 0.85\%. However, after the first three weeks, the Ins release from control was almost zero. In the case of DC loaded MS, the Ins release kinetics were nearly zero-order with a 1.9\% daily Ins release for at least one month. These findings indicate that the sizes of Ins and the DC are not the only factors that affect Ins release kinetics in PLGA. The lack of Ins release from control might be caused by Ins aggregation or insolubilization, which can be induced by PLGA degradation in an acidified microenvironment.

CONCLUSION

The star-shaped block copolymer and Ins form a dual-interaction complex (DC) comprised of ionic and hydrophobic interactions at pH 7.4. When tested in various NaCl concentrations, this complex showed significant tolerability against high salt-induced dissociation. When DC was incorporated into the PLGA MS, DC was evenly distributed in the MS. Unlike Ins-loaded PLGA MS, the Ins release profile of DC-incorporated PLGA MS was improved with a smaller initial burst, nearly zero-ordered release kinetics, and complete Ins release. In STZ-induced diabetic rats, DC-loaded PLGA MS controlled blood-glucose levels and maintained lower glucose levels without a loss of body weight compared to Ins-loaded PLGA MS. In conclusion, DC in PLGA MS shows high potential in applications of long-term sustained protein delivery.

REFERENCES


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