Development of a Novel Oral Insulin Delivery System Using Stimuli-responsive Polymers

Yong-Hee Kim

Biomedical Research Center, Korea Institute of Science and Technology

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Early studies on the drug delivery system (DDS) focused on the development of sustained release dosage forms to achieve longer lasting drug effects. Zero-order release kinetics have been attempted by changing device geometry, using controlling membranes, utilizing osmotic pressure, and/or using biodegradable materials. Recent advances in recombinant DNA technology and biotechnology produced many biologically active therapeutic peptides, polypeptides, and proteins. For these macromolecular therapeutic agents, new types of DDS are needed to deliver them to the target site and release them when required.

In very recent years, stimuli-sensitive(responsive) polymers and hydrogels which change their swelling in response to changing environmental variables, such as pH (1,2), temperature (3-5), ionic strength (6), electric field (7,8), magnetic field (9), light (10), ultrasound (11), or chemicals (12) have been studied. They have been investigated for application such as DDS, enzyme and cell immobilization, solute separation, immunodiagnostic assays, and purification and partitioning in biotechnology (13-17). For example, hydrogels have been investigated for insulin releasing system in response to change in glucose level in the surrounding media (18). Electric current and ultrasound triggered release of drugs from hydrogels appears promising for the transdermal systemic or local delivery (19). Stimuli-induced drug delivery from hydrogels is mainly based on the changing permeability of hydrogels with swelling changes. Other mechanisms like reversible skin formation or gel squeezing have been also proposed (20,21). Of all responsive hydrogels and polymers, most attention has been focused on pH-and temperature-responsive gels. Temperature and pH offer the greatest possibilities for controlled drug delivery and biomedical applications.

The response function of stimuli-responsive hydrogels is based on the phenomenon that gels can undergo phase transitions as a result of changing external conditions. There exist two types of temperature responsive hydrogels. One type is a positive thermosensitivity that shows an increasing swelling with increasing temperature and the other one is a negative thermosensitivity that shows a decreasing swelling with increasing temperature. Negative thermosensitivity is observed with hydrogels having a lower critical solution temperature(LCST) in aqueous solutions. These polymers are soluble in water at low temperatures but collapse and phase separate from solution above LCST. Similarly, pH-sensitive polymers synthesized with either acidic or basic components demonstrate reversible swelling/deswelling in acidic or basic media.

For a successful polypeptide drug delivery system, an appropriate polymeric carrier should be designed to match the particular delivery appli-

cation needed for the drug. Problems associated with oral polypeptide drug delivery include poor absorption across the gastrointestinal mucosa, protection against enzymatic degradation, and protection from the acidic environment of the stomach. To improve the absorption of biologically active polypeptide drugs through mucosal membranes, utilization of formulation adjuvants, such as enzyme inhibitors, permeation enhancers and drug stabilizers have been reported (22). In addition, the role of polymeric drug carriers for oral delivery are noted to protect the peptide from enzymatic degradation and to provide controlled release of an incoporated formulation.

Stimuli-responsive polymeric drug delivery, especially using temperature responsive and pH responsive hydrogels, can be applied to enhance drug loading and provide protection for oral polypeptide delivery. These two properties can be combined by synthesizing crosslinked pH/temperature responsive hydrogels (23). This system will allow polypeptide or macromolecular drug loading at low temperature (high swelling). Once the system is dried, acidic pH of the stomach prevents the hydrogel from swelling (no drug release). When the device reaches the neutral pH of the intestine, polymer swelling will allow drug release. The main drawback in using crosslinked hydrogels is the drug loading process. Typically, drug loading into crosslinked hydrogels has been performed by a solvent sorption technique. This procedure often requires an organic solvent or water-solvent mixture to facilitate drug solublity and enhance polymer swelling. However, polypeptide drugs are usually insoluble in organic solvents and may lead to the denaturation. Overall, the solvent sorption technique for large molecule loading is considered as a time-consuming and an inefficient process hampered by passive diffusion, low loading due to the limited partitioning, drying problems, and drug waste.

In contrast to crosslinked hydrogels, this research utilized linear pH/temperature sensitive polymers for a novel approach to aqueous phase loading of polypeptide or protein drugs into a polymer matrix and to provide controlled release of the drugs in response to pH. The solubilities of these linear polymers are dependent on pH and temperature in aqueous solutions. Specifically, the polymer is soluble below its LCST and precipitate above its LCST, and the LCST is also a function of pH. To achieve a specific pH/temperature responsive swelling/solubility, it is necessary to incorporate a third component, a hydrophobic monomer, to adjust the LCST and optimize polymer swelling and drug release. The utility of these pH/temperature responsive terpolymers for polypeptide drug loading and release was examined by varying physical parameters for drug loading and the pH of the release media. In addition, the bioactivity and conformational change of a model polypeptide, insulin released from these stimuli-responsive hydrogels were assessed. Also, in this study, the effects of various classes of enhancers/stabilizers on gastrointestinal insulin absorption in normal rats were investigated for the purpose of developing oral insulin delivery system with pH/temperature responsive polymeric carriers.

Synthesis and Characterization of pH/Temperature Responsive Polymers

Linear terpolymers composed of N-isopropylacrylamide(NiPAAm) as a temperature component, butylmethacrylate(BMA) as a hydrophobic component, and acrylic acid(AA) as a pH component with varying feed ratio(NiPAAm/BMA/AA mole ratio=90/10/0, 89/10/1, 88/10/2, 87/10/3, 86/10/4, or 85/10/5) were synthesized by the free radical polymerization using 2, 2'-azobisisobutyronitrile(AIBN) as a free radical initiator.

The weight average molecular weights of the

Table I – Lower Critical Solution Temperatures(LCST)* of the pH/temperature Sensitive Polymers

Polymer(NiPAAm/BMA/AA)	LCST($^\circ$C)	
	pH 7.4	pH 2.0
90/10/0	13	14
89/10/1	15	14
88/10/2	16	14
87/10/3	20	14
85/10/5	31	14

*determined by turbidity at 450 nm

synthesized polymers determined by gel permeation chromatography were ranged from 48,000 to 75,000, with polydispersities of around 2.5.

The LCSTs of each polymer solution at two pHs(2 and 7.4) were determined by turbidity measurement and the results are summarized in Table 1. At pH 2, the LCSTs were constant at around 14°C, which was independent of the polymer composition. However, at neutral pH, the LCST increased with an increasing AA content. With 5 mole % of AA, the polymer showed a LCST about 31°C at pH 7.4, which is close to that of NiPAAm homopolymer.

Bead Fabrication With pH/Temperature Responsive Polymer

As shown in Figure 1, aqueous polymer solutions(5 or 10% w/v, pH 2.0, 4°C) containing insulin, polymer, and additives were dropped into mineral oil kept at a temperature above the LCST of the polymer solution using a syringe with a blunt-ended needle. The oil was covered with a layer of decane to reduce surface tension and to aid penetration of the solution drop at the air/oil interface. The formed beads were immediately filtered and washed with hexane to remove the residual oil on the bead surface, and then dried in a rotary evaporator with aspiration at room temperature.
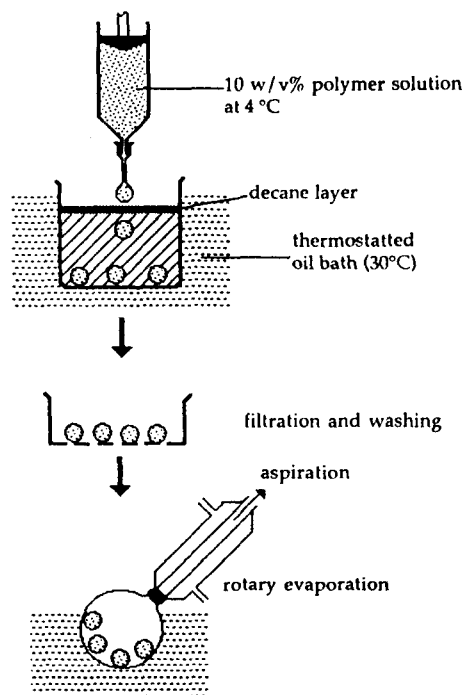
Macromolecule Loading and Release

Figure 1- Bead fabrication procedure.

Model solutes used were insulin, albumin, and FITC-dextran of various molecular weights. The loading efficiencies measured under various conditions are summarized in Table II. The solute loading efficiencies were not significantly changed within the tested polymer and drug concentrtations. The critical factors for high loading were found to be the ionic strength of aqueous solution and the bath temperature. Low molecular weight dextran(MW=4,400) showed almost 100% loading efficiency, while the loading efficiency decreased as increasing molecular weight of dextran. The loading efficiency of insulin increased as the ionic strength increased, reaching a maximum loading(about 90%) at the ionic strength of 0.25 and 30.

Insulin release patterns at neutal pH and 37°C, and at varying pH and 37°C were demonstrated in Figure 2. The insulin release in the media (isotonic phosphate-buffered solution) at pH 7.4 and 37°C was influenced by the content of acrylic

acid(AA) (Figure 2a). Without AA, less than 10% of the total insulin was released after 12 hours.

Table II–Solute Loading Efficiencies of pH/temperature sensitive Polymer* Beads

Ionic strength	Bath temp(℃)	Polymer conc.(% w/v)	Solute(0.5 ~1.0% w/v)	Loading efficiency(%)
0.05	40	5	Insulin	18 ± 4
			Albumin	17 ± 5
			FITC-Dextran 4.4 K	20 ± 5
			FITC-Dextran 9.4 K	22 ± 4
			FITC-Dextran 35.6 K	9 ± 3
0.05	30	5~10	Insulin	50 ± 9
			Albumin	55 ± 7
			FITC-Dextran 4.4 K	97 ± 5
			FITC-Dextran 9.4 K	35 ± 5
			FITC-Dextran 35.6 K	26 ± 4
0.25	30	10	Insulin	89 ± 8
			Albumin	85 ± 4
			FITC-Dextran 35.6 K	30 ± 6

*AA: 0~5 mole %

This release rate was slightly increased when 3 mole % of AA was incorporated to the polymers, while 5 mole % of AA dramatically enhanced the release rate, resulting in most of insulin being released within 8 hr. These results were correlated to the effect of AA on the LCST of coposimilar to that at constant pH 7.4 without changing pH of the release media.

Stability and Bioactivity of Insulin

The bioactivities of insulin released from the polymer beads *in vitro* and the recovered insulin were studied by measuring blood glucose level depression in male Sprague-Dawley rats. As presented in Figure 3, the blood glucose level depression activities of the released insulin at pH 7.4 and insulin recovered from the beads(5 mole % AA) kept in rat stomach for 5 hours were almost identical to the insulin standard solution. These results suggest that the mild conditions used for insulin loading into pH/temperature sensitive polymer beads are adequate in terms of loading efficiency and preservation of drug acitivity, and this polymeric carrier is able to protect the loaded
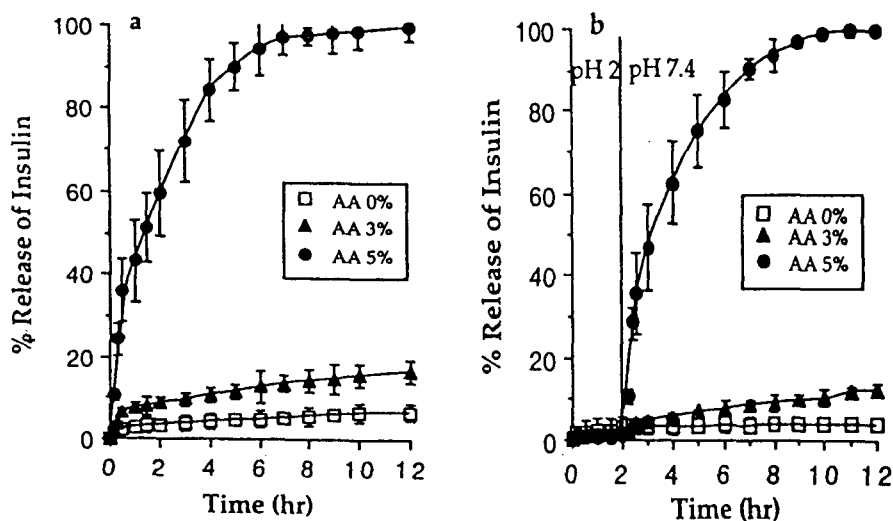

Figure 2—Effect of the acrylic acid(AA) content and pH on the release of insulin from the pH/temperature sensitive polymer beads : (a) at a constant pH 7.4(isotonic PBS) and 37℃(n=3, mean±s.d.) and (b) with varying pH 2 to 7.4.

drug(especially polypeptide drug) from the acidic condition of the stomach and gastric digestive enzymes. These results were further supported by CD spectra of insulin recovered from beads which were kept in the rat stomach. Apparently, there was no change in the conformation of the recovered insulin compared to the standard insulin.

Optimum Absorption Enhancer Formulation

The effects of various classes of enhancers/stabilizers on gastrointestinal insulin absorption in normal rats were investigated for the purpose of developing oral insulin delivery system with pH/temperature sensitive polymeric carriers. These compounds included steroidal surfactants (bile salts and bile acid derivatives), alkyl saccharides, nonionic surfactants, cyclodextrins, and mixtures of those.

lymers. The release rate was minimal at pH 2 regardless of the content of ionizable groups in the tested polymers(Figure 2b). The release profile at pH 7.4 following the release at pH 2.0 was

In vivo absorption study was performed in fasted normal rats with bovine insulin containing various amounts and types of enhancers instilled with an implanted catheter through stomach into duodenum. Among tested enhancers, steroidal surfactants were found to be the most effective in increasing insulin absorption. A more hydrophobic bile salt, sodium deoxycholate(NaDC) with optimum concentrations of 0.5~1.0%w/v showed large decreases in blood glucose level(Figure 4). Less hydrophobic bile salts(sodium taurocholate, sodium glycocholate, and sodium taurodeoxycholate) did not significantly enhance the intraduodenal(ID) insulin absorption at the same concentrations. The insulin solution containing NaDC prepared by first dissolving insulin and adding NaDC showed larger effects than that prepared by first dissolving NaDC and adding insulin that required 2 days at room temperature to become
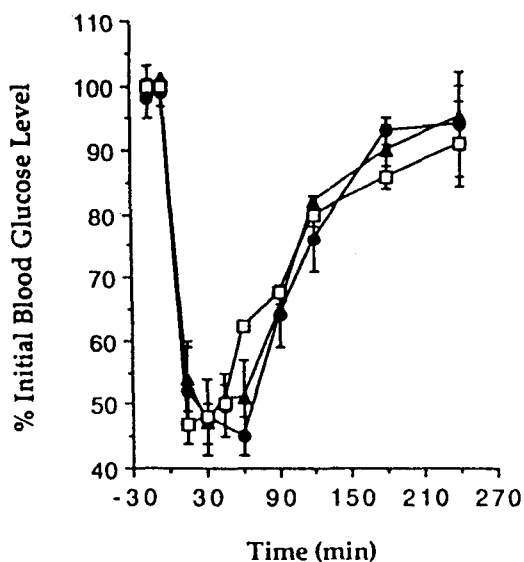
Figure 3—Blood glucose depression activity of insulin released from the pH/temperature sensitive polymer beads *in vitro* (open squares) and insulin recovered from the beads after keeping in rat stomach for 5 hours (closed triangles) and standard insulin (closed circles) (17.5 µg/m*l*/Kg, normal rat, i.v., n=3, mean ± s.d.).
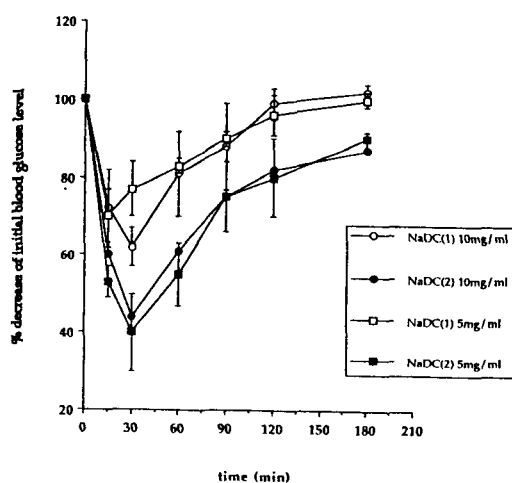
Figure 4—Effects of adding NaDC and insulin(1: NaDC-insulin and 2: insulin-NaDC) on blood glocose depression following ID administration of 150U/Kg insuling in normal rats.
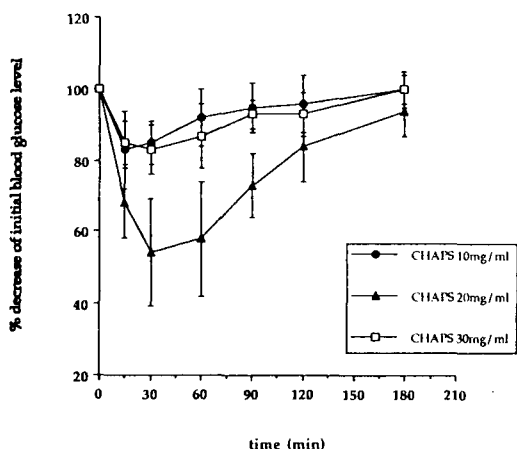
Figure 5 — Effect of CHAPS concentration on blood glucose depression following ID administration of 150U/Kg insulin in normal rats.

clear solutions. Coadministration of NaDC with some Pluronics or dodecyl maltoside(DDM) reduced the enhancing potency of NaDC. Of the bile acid derivatives studied, a zwitterionic compound, CHAPS [3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonate] was the most effective enhancer at 2% concentration(Figure 5). Like NaDC, CHAPS at concentrations higher and lower than 2% was less effective. Among nonionic surfactants, DDM was shown to be an effective enhancer.

Acknowledgement

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