Development of Totally Synthetic Glucose Responsive Gel with Phenylboronic acid Derivative as Sensor Moiety

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Introduction

With an attempt to develop a glucose-responsive polymer gel for use as a self-regulated insulin delivery system to treat diabetes, particular attention was paid to unique ability of phenylborate derivatives to form a reversible, covalent bonding with glucose. We have previously prepared a gel composed of N-isopropylacrylamide and 3-acrylamidophenylboronic acid (NB10 gel) exhibiting a reversible volume change synchronized with a change in the glucose concentration, through which the sufficiently controlled and pulse-shaped release of insulin was achievable at 28°C in a pH 9.0.

This work is intended to provide a synthetic methodology to optimize the operational conditions of the system to be physiological. The approach involves the use of a newly synthesized phenylborate derivative [4-(1',6'-dioxo-2',5'-diaza-7'-oxamyl) phenylboronic acid: DDOPBA] possessing an appreciably low pKa as glucose sensing moiety. Evaluations of glucose dependent changes in the swelling degree of the resultant copolymer gels for varied pH and temperatures revealed a markedly improved sensitivity near the physiological conditions. Discussion will be presented in terms of the effect of the modulated copolymer gel structure on the glucose responsive behavior.

Experimental

NB10 gel and ND10 gel (bearing 10 mol% of AAPBA and DDOPBA, respectively) were prepared by radical polymerization in DMSO at 60°C for 24 h, using AIBN as an initiator and methylene-bis-acrylamide as a cross-linking agent. FITC-insulin was loaded into the gel by immersing the gel disc in 50 mL of each experimental buffer [0.1 M CHES (pH9, I=0.15) and 0.1 M phosphate (pH8, I=0.15)] solution, containing 130 mg/L of FITC-insulin at 4°C for 24h. The gel disc was then transferred to the same buffer adjusted to pH6 for 1 h to form a surface skin layer. Release experiment was conducted in 50 mL of each experimental-conditioned buffer solution, with varied glucose-concentrations. The fluorescence intensity of the solution at 520 nm (excitation wavelength: 495 nm) was monitored at given time intervals to determine the released amount of FITC-insulin (Mt) from the gel based on the calibration curve. The total amount of entrapped insulin (M) in the gel was defined as the cumulative amount of FITC-insulin released from the gel after 2 days.

Results and Discussion

Figure 1 shows FITC-insulin release profiles from NB10 and ND10 gels. In all cases, the release rate increases with increasing glucose concentration. In the absence of glucose, insulin release from NB10 gel was effectively shut-off under the conditions of pH 9 and 28°C, while the release rate drastically increases with increasing glucose-concentration higher than 1 g/L, which is a range of euglycemia. More rapid release rates of insulin from ND10 gel as compared to that from NB10 gel can be observed under the same conditions. This is consistent with a larger amount of charged borate anions of DDOPBA than that of AAPBA due to its relatively low pKa. The monomer-state pKa values were determined via potentiometric titrations, to be 7.8 and 8.2 for DDOPBA and AAPBA, respectively. Release of insulin from ND10 gel in the absence of glucose, however, was not completely shut-off under these conditions. Under the conditions of pH8 and 25°C, on the other hand, a more sufficiently glucose-dependent increase in the release rate of insulin was achieved for ND10 gel. Also, the release was effectively shut-off in the absence of glucose. NB10 gel showed only a slight increase in the release rate with increased glucose concentration. This is due to an insufficient amount of charged borate anions of AAPBA under these conditions. Thus it was demonstrated that, by the use of a novel phenylborate moiety with decreased pKa, our system becomes operational under the condition that is closer to that of physiology.
Insulin injection: currently conducted treatment for Insulin Dependent Diabetes Mellitus.

Problems in the treatment:
- significant burdens to patients
- difficulty in controlling dosage

For more advanced treatment:
- development of self-regulated (thus safer) insulin delivery system, making use of the phase transition behavior of polymer gels.

Concept of our approach:
- Insulin
- Increase in glucose conc.
- Decrease in glucose conc.
- Shrink
- Swell
- Off
- On
- Volume change
- Insulin release
Equilibrium between the uncharged (①:hydrophobic) and the charged (②:hydrophilic) forms can be shifted to the direction of increasing charged borates (②+ ③) through the complexation with glucose.

Glucose dependent change in the ratio between the uncharged (①) and the charged borates (②+③) affect on the amphiphilic polymer solubility, also, contributes to the osmotic pressure change by counterions, inducing a reversible volume transition.
**NB Gel**

**poly(NIPAAm-co-AAPBA) gel: NB gel**

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\text{N-Isopropylacrylamide (NIPAAm)} \quad \text{3-Acrylamidophenylbromonic acid (AAPBA)}
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**Insulin release from NB gel at 28°C, pH9**

- Sufficiently controlled, pulse-shaped response is achievable.
- Synthetic material (non-natural components such as enzyme) and thus highly stable in the human body with a reliable functionality.

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**Phase diagram for NB gel at 28°C, pH9**

The NB Gel system is not be able to function under the physiological pH and temperature conditions.

For the use of the system as an *in vivo* insulin-delivery device

Synthetic approach to enable the system to be operated under the physiological conditions (pH 7.4, 37°C)
Improvement of the Operational pH Condition

Stabilization of charged borate (decreasing the pKa value)

An introduced stronger electron-withdrawing type of substituent into the phenyl ring causes decreased charge density on boron atom, making the borates more acidic.

Linear relation between the Hammett substituent constant ($\sigma$) and the pKa values predicts the reduced pKa value of the p-carbamoyl-substituted phenylborate as low as 7.65.
The modified structure has achieved a phenylborate-containing monomer endowed with a lowered pKa.

Figure  Apparent pKa values of AAPBA and DDOPBA as a function of glucose concentration, as determined from potentiometric titration at 25°C.

AAPBA: 3-acrylamidophenylboronic acid
pKa=8.23

DDOPBA: 4-(1',6'-dioxso-2',5'-diaza-7'-oxamyl)phenylboronic acid
pKa=7.79

The modified structure has achieved a phenylborate-containing monomer endowed with a lowered pKa.
Modified Structure Effect on the Glucose-Responsive Behavior

AAPBA  
(m-amide)  
DDOPBA  
(p-carbamoyl)

Opened: no glucose  
Fixed: glucose conc. 5 g/L

Figure  Changes in the equilibrium swelling degrees (phase diagrams) of the PNIPAAm-based copolymer gels bearing different phenylborate moieties: gPNIP-A-10 (squares) and gPNIP-D-10 (circles), as a function of temperature in the presence (fixed: glucose concentration of 5g/L) and the absence (opened) of glucose, for various pH conditions: (a) pH7.4, (b) pH8 and (c) pH8.5.
Release Profiles of FITC-Insulin at 20°C, pH 8.0

**Figure** Release profiles of FITC-insulin from poly(NIPAAm-co-AAPBA)[90/10]gel at 20°C, pH 8.0 for various concentrations of glucose: (▲) 0 g/L, (■) 1 g/L, (♦) 3 g/L.

**Figure** Release profiles of FITC-insulin from poly(NIPAAm-co-DDOPBA)[90/10]gel at 20°C, pH 8.0 for various concentrations of glucose: (▲) 0 g/L, (■) 1 g/L, (♦) 3 g/L.
Sterical hindrance due to $\alpha$-methyl groups on the main chains of PNIPMAAm inhibits favorable association of the hydrophobic groups, causing an exhibition of the higher LCST than that of PNIPAAm.
Phase Diagrams for PNIPMAAm-based Gels at the Physiological pH

Figure Changes in the equilibrium swelling degrees (phase diagrams) of the PNIPMAAm-based copolymer gels bearing DDOPBA as a phenylborate moiety: gPNIPM-D-10 (squares) and gPNIPM-D-20 (circles), as a function of the temperature in the presence (fixed: glucose concentration of 5g/L) and the absence (opened) of glucose under the physiological pH of 7.4.
Glucose-Induced Volume Changes of gPNIPM-D Gels at the Physiological pH

Figure Series of glucose concentrations: (diamonds) 0.5g/L, (squares) 1g/L, (triangles) 3g/L, (circles) 5g/L, induced changes in the volume of gPNIPM-D-20 gel, all with the initial glucose concentrations of 0 g/L, as a function of time at (a) 20°C and (b) 25°C under the physiological pH of 7.4. The values (dt) are standardized with those of the initially equilibrated state (di) in the absence of glucose at each temperature.
Effect of Introduced Carboxyl Group of Methacrylic Acid (MAAc): Phase Diagrams for gPNIPM-D-M gel

**Figure** Changes in the equilibrium swelling degrees (phase diagrams) of the PNIPMAAm-based copolymer gels bearing 20 mol% of DDOPBA with the varied content of methacrylic acid: (squares) no methacrylic acid introduced (gPNIPM-D-20 gel), (triangles) methacrylic acid content of 2 mol%, (gPNIPM-D-M-20-2) (circles) methacrylic acid content of 5 mol% (gPNIPM-D-M-20-5), as a function of the temperature in the presence (fixed: glucose concentration of 5g/L) and the absence (opened) of glucose under the physiological pH of 7.4.
Glucose-Induced Volume Changes of gPNIPM-D-M-20-2 Gel at Physiological pH

Figure Series of glucose concentrations: (diamonds) 0.5g/L, (squares) 1g/L, (triangles) 3g/L, (circles) 5g/L, induced changes in the volume of gPNIPM-D-M-20-2 gel, all with the initial glucose concentrations of 0 g/L, as a function of time at (a) 25°C and (b) 30°C under the physiological pH of 7.4. The values (dt) are standardized with those of the initially equilibrated state (di) in the absence of glucose at each temperature.
Introduction of Carboxyl Group of 2-Carboxyisopropylacrylamide

Structural analogy (continuous chain of isoprorylacrylamide groups) with that of the PNIPAAm main chain

Maintained hydrophobic aggregation forces, free from the effect of ionized or hydrogen-bonded carboxyl groups, results in the preservation of a sharp transition.

Glucose-Induced Volume Changes of gPNIPM-D-C-20-7.5 Gel under the Physiological Conditions (pH7.4, 37°C)

Figure Changes in the equilibrium swelling degrees (phase diagrams) of the PNIPMAAm-based copolymer gels bearing 20 mol% of DDOPBA with the varied content of CIPAAm: (squares) no CIPAAm introduced (gPNIPM-D-20 gel), (triangles) CIPAAm content of 5 mol%, (gPNIPM-D-C-20-5) (circles) CIPAAm content of 7.5 mol% (gPNIPM-D-C-20-7.5), as a function of the temperature in the presence (fixed: glucose concentration of 5g/L) and the absence (opened) of glucose under the physiological conditions (pH7.4, 37°C).
Glucose-Induced Volume Changes of gPNIPM-D-C-20-7.5 Gel under the Physiological Conditions (pH7.4, 37°C)

**Figure** Series of glucose concentrations: (squares) 0.5g/L, (diamonds) 1g/L, (triangles) 3g/L, (circles) 5g/L, induced changes in the volume of gPNIPM-D-C-20-7.5 gel, all with the initial glucose concentrations of 0 g/L, as a function of time under the physiological conditions (pH 7.4, 37°C). The values (dt) are standardized with those of the initially equilibrated state (di) in the absence of glucose.
Conclusions

In order to improve the sensitivity of the phenylborate-based, glucose-responsive system under the physiological conditions, modifications were made to the molecular design that include:

1) use of DDOPBA which was designed to possess a lower pKa
2) use of PNIPMAAm which brings about a higher LCST than that of PNIPAAm
3) introduction of carboxyl groups by use of methacrylic acid or 2-carboxyisopropylacrylamide.

As a consequence of the combined molecular effects, a distinct sensitivity under the physiological conditions was accomplished.

We expect, the present totally-synthetic system, to be highly stable in the human body, which should have a great potential for the use as a self-regulated insulin-delivery system to treat diabetes with a reliable functionality.