International Journal of Pharmaceutics 392 (2010) 83–91

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Potential use of γ-cyclodextrin polypseudorotaxane hydrogels as an injectable sustained release system for insulin

Irhan Ibrahim Abu Hashim[a,b], Taishi Higashi[a], Takayuki Anno[a], Keiichi Motoyama[a], Abd-ElGawad Helmy Abd-ElGawad[b], Mohamed H. El-Shabouri[b], Thanaa Mohamed Borg[b], Hidetoshi Arima[a,*]

[a] Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan
[b] Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

ARTICLE INFO

Article history:
Received 18 December 2009
Received in revised form 20 February 2010
Accepted 10 March 2010
Available online 16 March 2010

Keywords:
Insulin
Cyclodextrin
Polypseudorotaxane
Hydrogel
Sustained release system

ABSTRACT

The development of injectable hydrogels for protein delivery is a major challenge. In this study, insulin/α-cyclodextrin (α-CyD) and γ-CyD polypseudorotaxane (PPRX) hydrogels were prepared through inclusion complexation between high molecular weight poly(ethylene glycol) (PEG) and CyDs. The α-CyD and γ-CyD PPRX hydrogels were formed by inserting one PEG chain in the α-CyD cavity and two PEG chains in the γ-CyD cavity. Insulin/CyD PPRX hydrogel formation was based on physical crosslinking induced by self-assembling without chemical crosslinking reagent. The supramolecular structures of insulin/CyD PPRX hydrogels were confirmed with ^{1}H nuclear magnetic resonance (^{1}H NMR), X-ray diffraction, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). The in vitro release study showed that the release rate of insulin from the CyDs PPRX hydrogels decreased in the order of γ-CyD PPRX hydrogel > α-CyD PPRX hydrogel. This decrease was controlled by the addition of CyDs to the medium. The serum insulin level after subcutaneous administration of γ-CyD PPRX hydrogel to rats was significantly prolonged, accompanying with an increase in the area under serum concentration–time curve, which was clearly reflected in the prolonged hypoglycemic effect. In conclusion, these results suggest the potential use of γ-CyD PPRX hydrogel as an injectable sustained release system for insulin.

1. Introduction

During the past decade, novel polymeric microspheres, polymer micelles, and hydrogel-type materials have been shown to be effective in enhancing drug targeting specificity, lowering systemic drug toxicity, improving absorption rates, and providing protection for pharmaceuticals against biochemical degradation. These are all goals of drug delivery (Varshosaz, 2007).

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (Peppas et al., 2000), and are favored in a broad range of pharmaceutical and biomedical applications (Fedorovich et al., 2007; Hoffman, 2002; Peppas, 1997; Van Tomme and Hennink, 2007). Particularly, great interest has been focused on hydrogels for delivery of delicate protein and peptide drugs (Bromberg and Ron, 1998; Heller, 1993; Kissel et al., 2002). Both natural and synthetic polymers can be used for the production of hydrogels. Crosslinking of the polymer chains can be achieved by various chemical or physical crosslinking methods (Hennink and Van Nostrum, 2002). Conventional chemically crosslinked hydrogels are often applied as implantables, and the incorporation of drugs by solution sorption may limit the loading level and be time consuming. In addition, the covalent crosslinking reaction may conjugate the drug to the hydrogel or impair the chemical integrity of drugs. The hydrogel also may become non-biodegradable, with ill-defined composition. Therefore, a drug delivery formulation formed by physical hydrogels, where gelation and drug loading can be achieved simultaneously in an aqueous environment without chemical crosslinking, would be attractive (Li et al., 2003). So far, various approaches have been exploited to develop physical polymer hydrogels from different polymers, including gelation in response to temperature or pH change (Chen and Hoffman, 1995), hydrophobic interaction (Jeong et al., 1997), crystallization (Stenekes et al., 2001) and ionic interaction (Wang et al., 2004), or based on the complexation of enantiomeric polymer or polypeptide segments (Li and Vert, 2003; Nowak et al., 2002). Poly(ethylene glycol) (PEG) is a non-toxic, water soluble polymer which resists recognition by the immune system. It exhibits rapid clearance

* Corresponding author. Tel.: +81 96 371 4160; fax: +81 96 371 4420.
E-mail address: arimah@gpo.kumamoto-u.ac.jp (H. Arima).

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doi:10.1016/j.ijpharm.2010.03.026

from the body, and has been approved for wide range of biomedical applications. Because of these properties, hydrogels prepared from PEG are excellent candidates as biomaterials (Peppas et al., 1999).

Recently, supramolecular assemblies have attracted a great attention, due to their intriguing topologies and their applications in various fields such as nanodevices, sensors, molecular switches, and drug delivery systems, etc. Macrocyclic compounds are most often used as host molecules in supramolecular chemistry, of which cyclodextrins (CyDs) have been widely applied to drug delivery system because of their good bioadaptability (Uekama, 1999; Uekama et al., 1998). CyDs are cyclic oligosaccharides composed of six (α-CyD), seven (β-CyD), and eight (γ-CyD) glucopyranose units that can form inclusion complexes with various organic and inorganic compounds (Saenger, 1980). Harada et al. first reported the supramolecular assemblies of PEG and α-CyD, in which a number of the cyclic molecules are spontaneously threaded onto the polymer chain (Harada and Kamachi, 1990; Harada et al., 1992). Subsequently, gelation is induced by physical crosslinking between the inclusion complexes through hydrogen bonding between the CyDs along the PEG chains (Choi et al., 2002; Huh et al., 2001). These complexes are called polypseudorotaxane (PPRX), because the CyDs can be dethreaded of the polymer chain when dissolved in water. This complexation shows the size-dependency, i.e. α-CyD and γ-CyD form the PPRX with one PEG chain and two PEG chains, respectively, while β-CyD forms the PPRX with poly(propylene glycol).

Insulin secreted by the β-cells of the pancreatic islets is the primary hormone responsible for controlling the transport, utilization and storage of glucose in the body. Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by pancreas, or by the ineffectiveness of the insulin produced. For over than 80 years, injection or infusion of insulin into the subcutaneous tissue has been the major route of insulin delivery used in clinical practice. This mimics, as close as possible, secretion of insulin by healthy pancreas. Recently, inhalable insulin formulation (Exubera™) was on market, but unfortunately, it could not be proven to be more clinically or cost effective than existing treatments. A limitation of the parenteral route for delivery of peptides and proteins is the extremely short half-lives of these drugs that demands repeated administration which is inconvenient to the patient (Mccarthy, 2004). To decrease injection frequency of basal insulin supply, several research groups have investigated the potential use of biodegradable polymers as a sustained-release carrier (Barichello et al., 1999; Choi and Kim, 2003; Kim et al., 2001; Takenaga et al., 2002). Previously, we found that the pegylated insulin forms PPRX with α- and γ-CyDs in a similar manner as PEG does, and the resulting PPRX may be useful as one of sustained drug delivery techniques of pegylated insulin (Higashi et al., 2007, 2008, 2009). However, this approach required pegylation of insulin. To develop non-pegylated insulin application for sustained release system, in this article, we report the detailed studies on in vitro and in vivo evaluations of α- and γ-CyD PPRX hydrogels for insulin delivery.

2. Materials and methods

2.1. Materials

Bovine insulin (28.7 IU/mg) was purchased from Sigma–Aldrich (St Louis, MO). PEG (M.W. 20,000) was obtained from Katayama Chemical Co. (Osaka, Japan). CyDs were donated by Nihon Shokuhin Kako (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade and deionized double distilled water was used throughout the study.

2.2. Hydrogel formation

Insulin/CyD PPRX hydrogels were prepared by dissolving insulin (0.5 μmol, 2.87 mg) in 0.1N HCl then added to 0.5 mL of an aqueous solution of PEG with a typical concentration of 100 mg/mL. The prepared insulin-PEG solution was mixed with 3.35 mL of an aqueous α-CyD (145 mg/mL) or 2.23 mL of an aqueous γ-CyD (232 mg/mL) solution. The mixture was kept in a refrigerator at 4 °C for 12 h to form a viscous gel.

2.3. ^{1}H NMR spectroscopic studies

^{1}H NMR spectra were taken at 25 °C on a JEOL JNM-ECP500 spectrometer (Tokyo, Japan) operating at 500 MHz, using a 5-mm sample tube. Dried α- and γ-CyDs PPRX hydrogels (1 mg) were dissolved in 600 μL of deuterated DMSO (DMSO-d_6). The DMSO signal was used as an internal reference for ^{1}H NMR. Chemical shifts were expressed in parts per million (ppm) relative to that of the DMSO signal (2.49 ppm). To calculate the stoichiometry of the CyDs PPRX, the integral values of the anomeric proton (4.8 ppm) of CyDs and those of the ethylene protons (3.5 ppm) of the PPRX were compared.

2.4. Powder X-ray diffraction studies

Powder X-ray diffraction patterns of the dried samples of supramolecular hydrogels and its individual components were measured by a Rigaku RINT 2500 VL X-ray diffractometer (Tokyo, Japan) with a Ni filtered CuKα radiation, a voltage of 40 kV, a current of 40 mA, a scanning speed of 1°/min, a time constant of 2 s, a scan range of 2θ = 5–35°, a divergent slit of 1.74 mm (1°), a scattering slit of 0.94 mm (1°) and a receiving slit of 0.15 mm.

2.5. Differential scanning calorimetric (DSC) studies

The DSC analysis was carried out using a DSC8240 (Rigaku, Tokyo, Japan). A sample weight of 5 mg was heated in aluminum pan at a heating rate of 20 °C/min from 25 to 175 °C under nitrogen atmosphere.

2.6. Scanning electron microscope (SEM) studies

The surface morphology of the hydrogels, and their physical mixtures was studied using a scanning electron microscope (JSM-5800, JOEL, Tokyo, Japan). Before SEM observation, specimens of the dried samples were fixed on aluminum stubs and coated with gold for 10 s (30 mM, 8 Pa) by the JFC-1200 Fine Coater (JOEL, Tokyo, Japan).

2.7. Swelling ratio measurement

The swelling ability of insulin/α- and γ-CyD PPRX hydrogels was determined gravimetrically (Koo et al., 2009). The dried weight (Wd) of the lyophilized sample was measured (initial weight was 100 mg). The sample was added to distilled water. The swollen weight (Ww) of the insulin hydrogel was determined at given time intervals. The swelling ratio (q) of the α- and γ-CyD PPRX hydrogels was calculated from the following equation q = (Ww − Wd)/Wd (Xu et al., 2006).

2.8. In vitro release of insulin from CyD PPRX hydrogels

The in vitro release rate was measured by the modified dispersed amount method (Higashi et al., 2008). Insulin/CyD PPRX hydrogels containing 0.5 μmol insulin were diluted to a total volume

of 10.0 mL with phosphate-buffered saline (pH 7.4, PBS) containing various CyD concentrations (α-CyD system: 0, 10, 40, 73 or 145 mg/mL and γ-CyD system: 0, 20, 80, 116 or 232 mg/mL). The samples were incubated at 37 °C with mild shaking. At certain time intervals, an aliquot of the dissolution medium was withdrawn and replaced with the same volume of the fresh CyD solution. The collected samples were filtered using membrane filters (cellulose acetate 0.45 μm, DISMIC®-25 CS, Toyo Roshi, Tokyo, Japan) and analyzed for insulin by high performance liquid chromatography (HPLC) (De Rosa et al., 2005; Quaglia et al., 2003). The HPLC system consisting of a L-7100 pump, an L-7200 autosampler, a L-7400 UV detector and a D-7500 integrator was used. The quantitative analysis was performed by reverse-phase chromatography on a CAPCELL PAK 5-μm C18 column (4.6 mm × 250 mm) (Shiseido, Tokyo, Japan). The mobile phase was a mixture of water and acetonitrile (7:3, v/v) containing 0.1% (v/v) of trifluoroacetic acid (TFA). The flow rate was 1 mL/min and the detection wavelength was 280 nm. In vitro release study of insulin/PEG/CyDs solutions in 10 mL PBS (pH 7.4) was also performed in comparison with that of the CyDs PPRX hydrogels.

2.9. Subcutaneous administration of insulin/α- and γ-CyD PPRX hydrogels to rats

Serum insulin and glucose levels of rats were measured as follows: insulin (3.0 IU/kg) solution, insulin/PEG/CyD solution in PBS (pH 7.4) or insulin CyDs PPRX hydrogels in PBS containing (145 or 232 mg/mL) of α- or γ-CyD, respectively, was subcutaneously injected in male Wistar rats (200–250 g). Animals were fasted for 12 h before dosing. At appropriate time intervals, blood samples were taken from the jugular veins. Serum insulin and glucose levels were determined by enzyme immunoassay using Glyzyme Insulin-EIA Test Wako (Wako Pure Chemicals, Osaka, Japan) and the mutarotase-glucose oxidase method using Glucose-CII-Test Wako (Wako Pure Chemicals, Osaka, Japan), respectively. Serum glucose levels were expressed as a percentage of the initial glucose level before injection.

2.10. Statistical analysis

Data are given as the mean ± S.E. Statistical significance of means for the studies was determined by analysis of variance followed by Scheffe's test. *p*-Values for significance were set at 0.05.

3. Results and discussion

3.1. Formation of CyDs PPRX hydrogels

Fig. 1A shows the solutions after mixing the insulin/PEG solution with α-, β- and γ-CyDs solutions and standing for 12 h at 4 °C. The α- and γ-CyD solutions gave hydrogels, whereas the β-CyD solution did not form any hydrogels, indicating the formation of

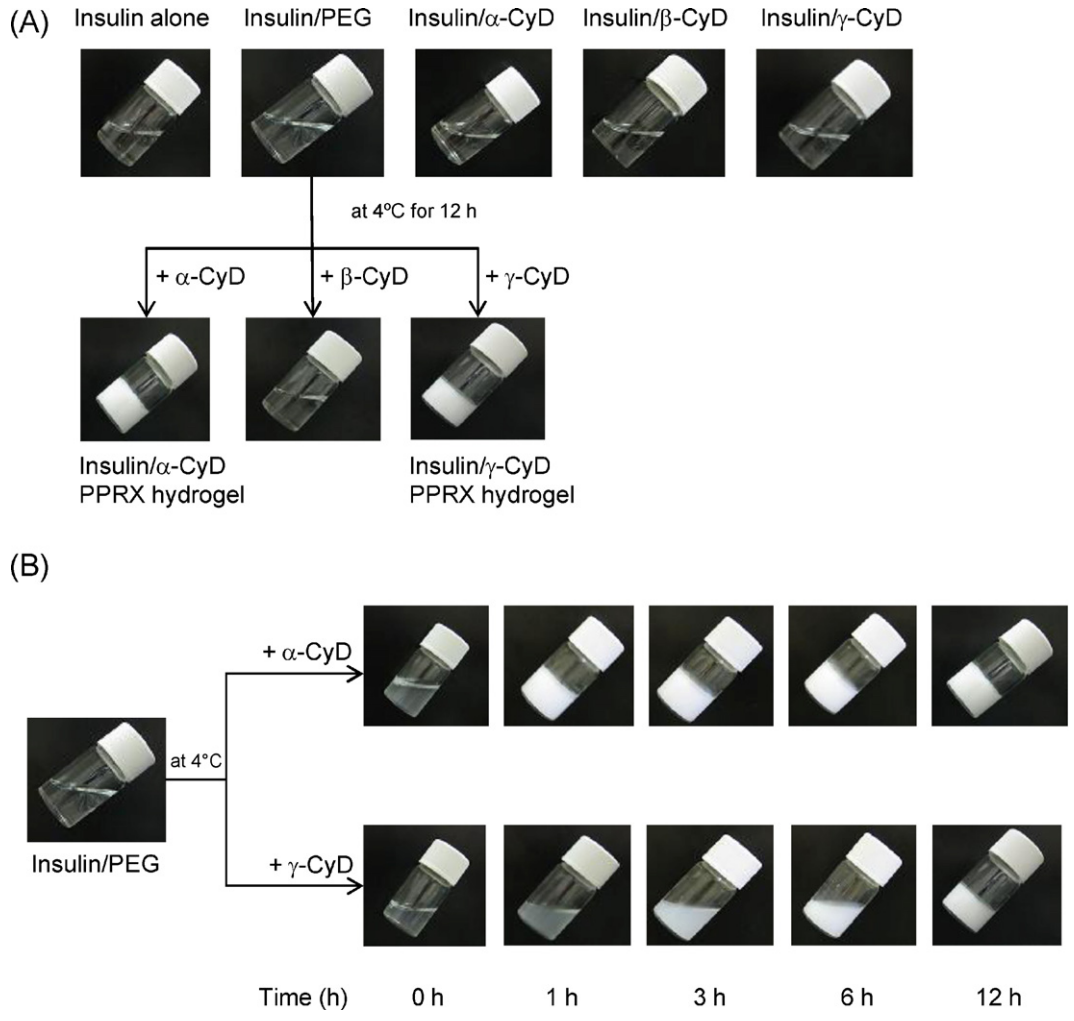

Fig. 1. Optical Photographs of insulin/CyD PPRX Hydrogels. The prepared insulin-PEG solution was mixed with aqueous CyD solutions. (A) The mixture was placed at 4 °C for 12 h. (B) The effect of the incubation time on the formation of the hydrogel.

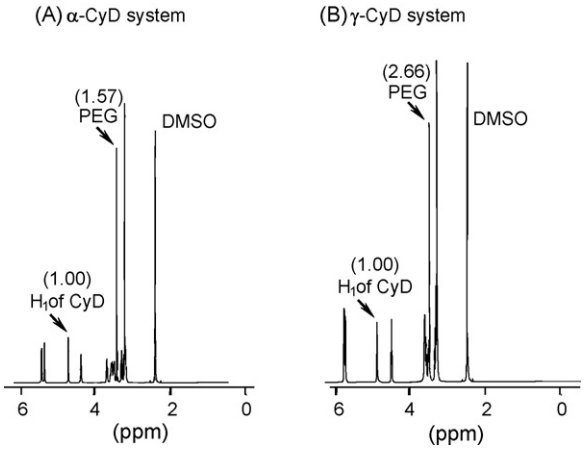

Fig. 2. ^{1}H NMR spectra of (A) insulin/α-CyD and (B) insulin/γ-CyD PPRX hydrogels in DMSO-d_6 at 25 °C.

insulin/α- and γ-CyDs PPRX hydrogels. As reported before, PEG chains with a molecular weight higher than 2000 can be threaded by α-CyD to form supramolecular self-assembly gelatinous complexes, where the PEG chains are partially included by α-CyD cavities in which only physical crosslinking is involved (Li et al., 1994). Meanwhile, Harada et al. have reported the formation of self-assembly of double-stranded inclusion complexes of PEG with γ-CyD, in which two polymer chains are threaded through the macrocycles (Harada et al., 1994). On the other hand, β-CyD did not form complexes with PEG, since a PEG chain is too slim and two PEG chains are too thick to fit in the β-CyD cavity (Harada, 1996). When α- and γ-CyD were added to the insulin/PEG solutions, the solutions became opaque within several minutes and finally changed to gel (Fig. 1B). The induction time for gelation ranged from several minutes to several hours, depending on the type of the CyD added.

3.2. ^{1}H NMR spectroscopy

The stoichiometry of the PPRX was accurately determined by measuring peak areas of the anomeric proton of CyDs and the ethylene protons of the insulin/CyD PPRX hydrogels in ^{1}H NMR spectra after dissolving the solid PPRX in DMSO-d_6 (Fig. 2). The results indicated that 192.7 and 85.5 moles of α- and γ-CyDs, respectively, were involved in the PPRX formation, i.e. the coverage of the PEG chain by α- or γ-CyD was 87.6% or 77.7% (Table 1), respectively, when assumed that two (ethylene glycol) repeat units are included in one CyD cavity (Harada and Kamachi, 1990; Harada et al., 1994). These results suggest that α-CyD forms PPRX with one PEG chain, while γ-CyD forms PPRX with two single or separate PEG chains, as reported previously in the pegylated insulin with CyD systems (Higashi et al., 2009).

Table 1
Yields and compositions of insulin/CyD PPRX hydrogels.

Protein	CyD	Yield (%)	$CyDs_{number}$[a]	Coverage[b] (%)
Insulin/PEG	α-CyD	58.5 ± 3.12	192.7 ± 3.87	87.6 ± 1.76
	γ-CyD	70.4 ± 8.27	85.5 ± 1.15	77.7 ± 1.04

Each value represents the mean ± S.E. of three experiments.
[a] Number of CyDs units involved in the polypseudorotaxane formation with one PEG chain.
[b] Coverage = 2 (CyDs per PEG)/(PEG repeat units), assuming that CyDs include 2 PEG repeat units.

3.3. X-ray diffraction patterns

The threading of CyDs onto the PEG chains and formation of inclusion complexes and their supramolecular self-assembly in the hydrogels were confirmed by X-ray diffraction patterns (Fig. 3). In the preparation of the physical mixtures, the amounts of α-CyD and γ-CyD (535.2 and 356.7 mg, respectively) were much higher than that of PEG (50 mg) which might have an influence on the appearance of PEG peaks in the X-ray diffraction pattern. The X-ray diffraction patterns of insulin/CyD PPRX hydrogels were different from those of physical mixtures, but the same as those of CyD PPRX hydrogels (Harada, 1996). In particular, the sharp reflection at $2\theta = 19.8^\circ$ ($d = 4.46$ Å) is a characteristic peak for the channel-type of α-CyD inclusion complexes (Li et al., 2001). This suggests that the gel system contains the necklace-like inclusion complex formed by α-CyD threading onto parts of the PEG segments, and its self-assembling of PEG and α-CyD acts as a physical crosslinking, providing the primary driving force for the gelation of the solutions of α-CyD and the high molecular weight of PEGs (Li et al., 2003). In the case of insulin/γ-CyD PPRX hydrogel in comparison to plain γ-CyD PPRX hydrogel, both samples showed less crystallinity than α-CyD PPRX hydrogels. This less crystallinity could be due to the more complex structure of the formed γ-CyD PPRX hydrogel as two PEG chains can be included within the γ-CyD cavity through

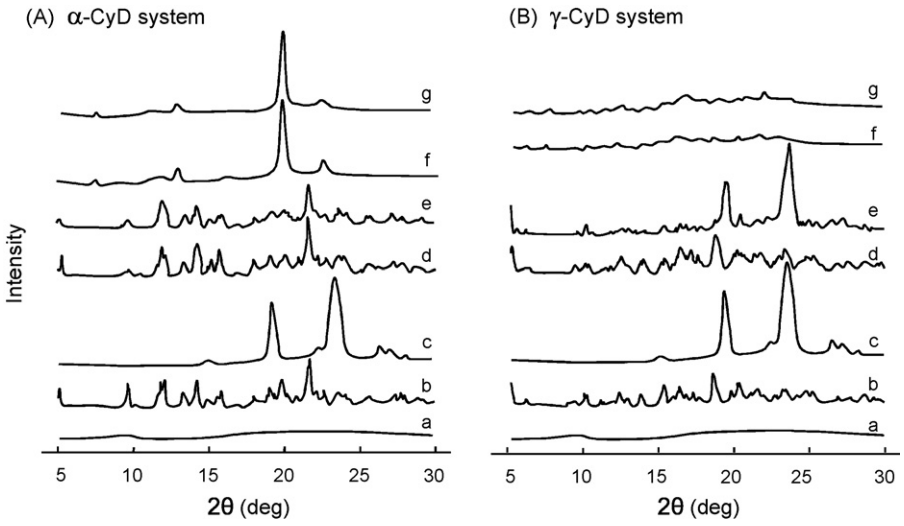

Fig. 3. Powder X-ray diffraction patterns of (A) insulin/α-CyD and (B) insulin/γ-CyD PPRX hydrogels. (a) insulin alone; (b) CyD alone; (c) PEG alone; (d) PEG/CyD physical mixture; (e) insulin/PEG/CyD physical mixture; (f) plain CyD PPRX hydrogels; (g) insulin/CyD PPRX hydrogels.

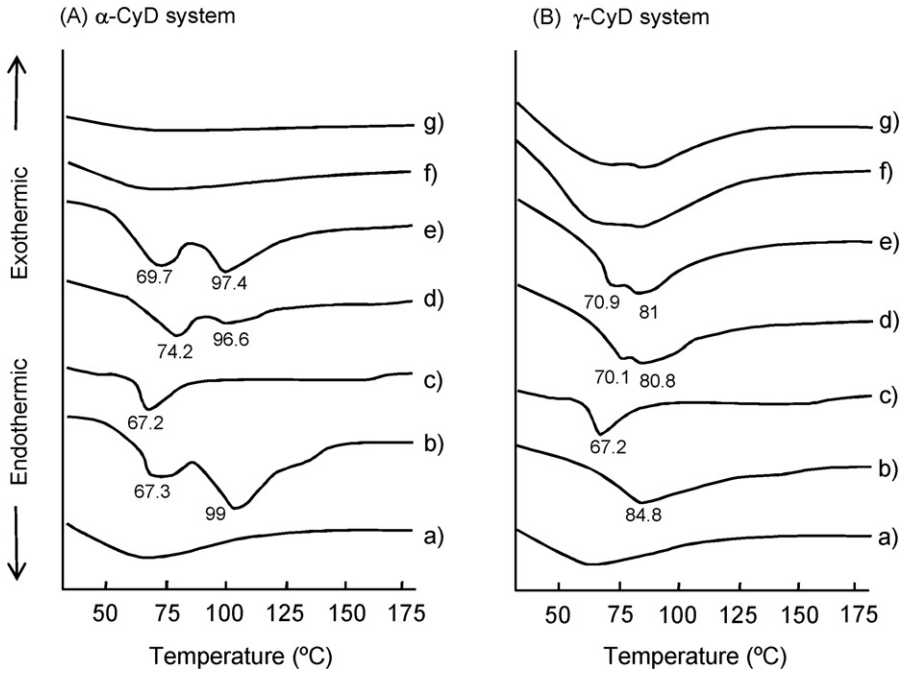

Fig. 4. DSC thermograms of (A) insulin/α-CyD and (B) insulin/γ-CyD PPRX hydrogels. (a) insulin alone; (b) CyD alone; (c) PEG alone; (d) PEG/CyD physical mixture; (e) insulin/PEG/CyD physical mixture; (f) plain CyD PPRX hydrogels; (g) insulin/CyD PPRX hydrogels.

an intermolecular and intramolecular complex formation (Harada et al., 1994).

3.4. Differential scanning calorimetric (DSC) studies

To confirm the formation of CyD PPRX hydrogels, we measured the DSC thermographs (Fig. 4). Here we confirmed that the results showed no difference in the DSC thermograms at the 10 °C/min and 20 °C/min. So, we selected 20 °C/min to measure promptly them. The endothermic peaks of α-CyD, γ-CyD or PEG did not completely disappear in the corresponding to PEG/CyDs physical mixture. However, the endothermic peaks of α-CyD and PEG in the α-CyD PPRX hydrogel system completely disappeared. These results suggest that α-CyD PPRX hydrogel was prepared probably

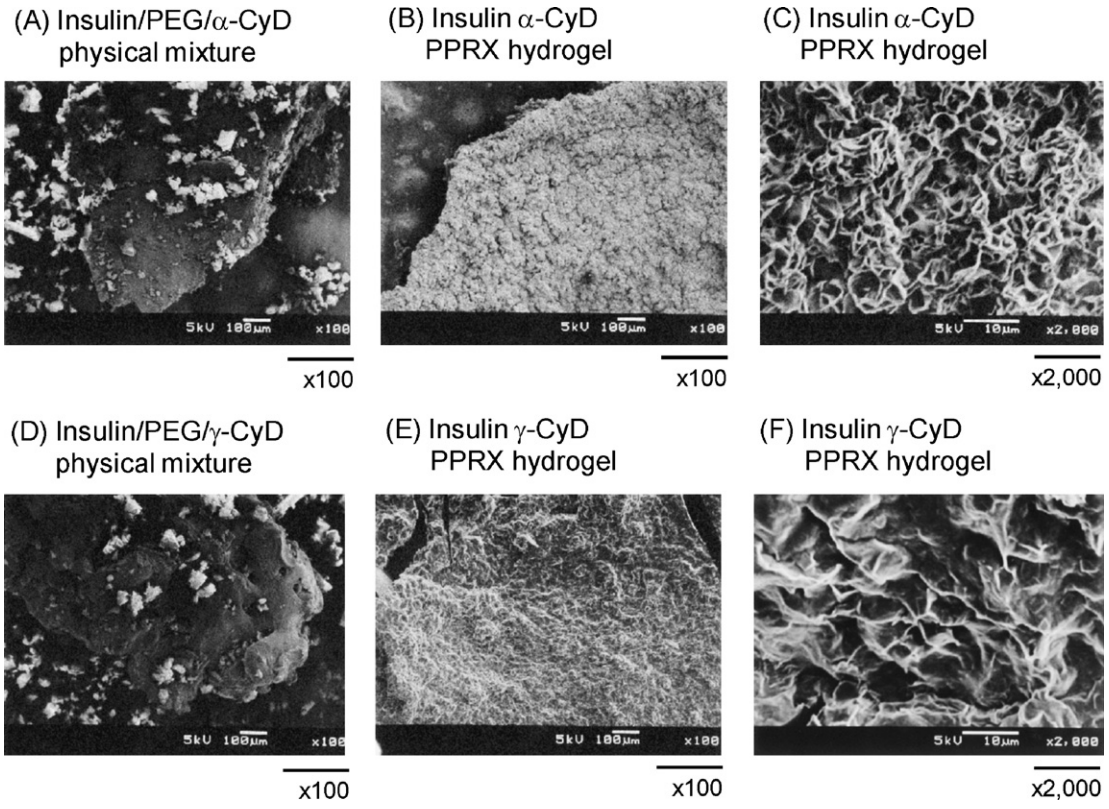

Fig. 5. SEM micrographs of insulin/CyD PPRX hydrogels.

by the formation of PPRX (Fig. 4A). Similar results were observed in the γ-CyD PPRX hydrogel system (Fig. 4B). Comparing between plain CyD PPRX hydrogels and those containing the drug, no difference was observed in their DSC thermograms, indicating that there is no interaction between insulin and the other components of the hydrogel. This could be due to the entrapment of insulin in the physically crosslinked hydrogel system.

3.5. Scanning electron microscope (SEM) studies

The morphology of the supramolecular hydrogels was completely different from its physical mixtures as observed by a SEM (Fig. 5). The micrographs of both α-CyD and γ-CyD PPRX hydrogels showed a porous structure in agreement with the formation of polymer network, and it could help to form a high water content hydrogel as is generally shown in other hydrogels (Crescenzi et al., 2007).

3.6. Swelling ratio

The supramolecular hydrogels have porous structure, so it can interact with the aqueous medium. The swelling kinetics profile of the hydrogels is shown in Fig. 6. The hydrogels showed high water-swelling ability in distilled water. After 1 h, insulin/α-CyD and insulin/γ-CyD PPRX hydrogels can absorb about 3.6 and 1.3 times their mass of water, respectively. These results indicate that α-CyD PPRX hydrogel has higher swelling ratio than γ-CyD PPRX hydrogel. This may be explained by the difference in the porous structures of the hydrogels. This phenomenon may be due to the fact that a more porous network structure makes water easier to diffuse into or out of the matrix (Zhang et al., 2001).

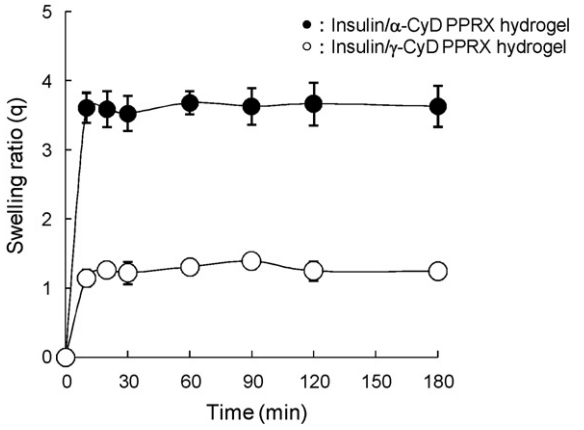

Fig. 6. Swelling ratio of insulin/α-CyD and insulin/γ-CyD PPRX hydrogel systems in distilled water. Each point represents the mean ± S.E. of three experiments.

3.7. In vitro release study

Since the formation of hydrogels is induced by the supramolecular self-assembling in aqueous solution, bioactive agents like insulin could be physically entrapped in the hydrogel and its

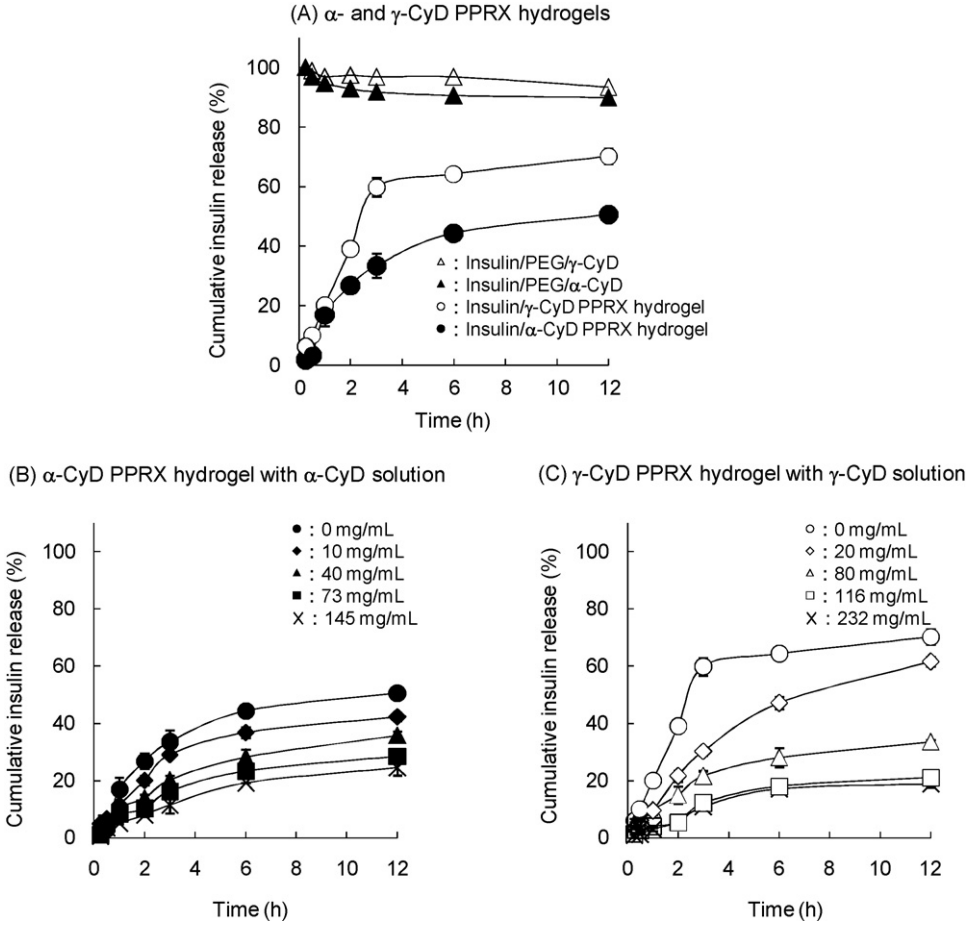

Fig. 7. In vitro release profiles of insulin in PBS (pH 7.4) from insulin/CyD PPRX hydrogel systems. (A) Insulin/α- and γ-CyD PPRX hydrogels, (B) insulin/α-CyD PPRX hydrogel at various concentrations of α-CyD solution, (C) insulin/γ-CyD PPRX hydrogel at various concentrations of γ-CyD solution. Each point represents the mean ± S.E. of three experiments.

Table 2
In vivo pharmacodynamic parameters of insulin/PEG/CyDs solution and insulin/CyD PPRX hydrogel systems.

Sample	T_{nadir} (h)[a]	C_{nadir} (%)[b]	AUC_G (%h)[c]	MRT_G (h)[d]
Insulin	2	42.7 ± 2.70	340.9 ± 24.3	4.7 ± 0.15
Insulin/PEG/γ-CyD solution	2	40.3 ± 2.04	345.6 ± 15.4	4.6 ± 0.21
Insulin/PEG/α-CyD solution	2	40.9 ± 1.47	352.5 ± 25.7	4.7 ± 0.22
Insulin/γ-CyD PPRX hydrogel	4*	38.5 ± 1.59*	437.5 ± 10.9*	5.3 ± 0.06
Insulin/α-CyD PPRX hydrogel	2	58.2 ± 4.24*	201.6 ± 26.1*	3.8 ± 0.34

Each value represents the mean ± S.E. of ten experiments.
[a] Time to nadir serum glucose levels.
[b] Nadir serum glucose levels.
[c] The cumulative percentage of change in serum glucose levels up to 12 h post-administration.
[d] Mean reduced time of serum glucose levels.
* $p < 0.05$ versus insulin.

in vitro release properties was evaluated. Fig. 7A shows the release profiles of insulin/CyD PPRX hydrogels in PBS (pH 7.4) at 37 °C. The release rates of insulin from insulin/PEG/CyDs solutions were very rapid, compared to the supramolecular hydrogels. In addition, the release rate of insulin from the supramolecular hydrogels decreased in the order of γ-CyD PPRX hydrogel > α-CyD PPRX hydrogel. The release rate of insulin from its hydrogels was decreased by the addition of CyDs in the medium (α-CyD system: 0 mg/mL > 10 mg/mL > 40 mg/mL > 73 mg/mL > 145 mg/mL and γ-CyD system: 0 mg/mL > 20 mg/mL > 80 mg/mL > 116 mg/mL > 232 mg/mL) as shown in Fig. 7B and C. This could be due to the threading and de-threading of PPRX which are in equilibrium with free host and guest molecules (Harada, 1996). These results indicate that the release rates of insulin from PPRX supramolecular hydrogels can be controlled by adjusting CyD concentration in the medium. Presumably, erosion of the gel through de-threading of the PEG chains from the CyD cavities contributes to the drug release. A potential advantage of this feature is that the release kinetics might be less dependent on the properties of the drugs, hence rendering the delivery system more widely applicable to different drugs (Li et al., 2003).

3.8. Subcutaneous administration of insulin/α-CyD and γ-CyD PPRX hydrogels to rats

Next, we investigated the effects of CyD PPRX hydrogels on serum glucose and insulin levels in vivo. Fig. 8 shows the serum glucose level-time profiles after subcutaneous administration of insulin, insulin/PEG/CyDs or insulin/α-CyD and γ-CyD PPRX hydrogels in the presence of CyDs in PBS (pH 7.4) to rats. When insulin, insulin/PEG/CyDs solution or insulin α-CyD PPRX hydrogel was injected, the minimal glucose level occurred at about 2 h after injection and then the serum glucose levels were recovered within 6 h to the basal level. This glucose profile of insulin was qualitatively similar to that reported by Higashi et al. (2008) and Takeuchi et al. (1996), although the dose of insulin was different (3 IU/kg insulin versus 2 IU/kg and 4–4.5 IU/kg, respectively). On the other hand, insulin γ-CyD PPRX hydrogel significantly sustained the hypoglycemic effect where the serum glucose level was recovered within 12 h after administration. Table 2 shows the pharmacodynamic parameters after subcutaneous administration to rats. The area under the serum glucose level-time curve (AUG_G) and mean reduced time of serum glucose levels (MRT_G) were significantly increased by the administration of insulin/γ-CyD PPRX hydrogel. The difference in the hypoglycemic effect of insulin/γ-CyD PPRX hydrogel from that of insulin/α-CyD PPRX hydrogel could be due to the safety profile of γ-CyD such as low hemolytic activity and superior biodegradability, compared to those of α-CyD (Higashi et al., 2008; Irie and Uekama, 1997).

Fig. 9 and Table 3 show the serum insulin level-time profiles and pharmacokinetic parameters after subcutaneous administration of insulin or insulin/γ-CyD PPRX hydrogel in the presence of γ-CyD in PBS (pH 7.4) to rats. When insulin was injected alone, the time (T_{max}) required to reach the maximum level (C_{max}) of insulin occurred at 0.5 h after injection and then the serum insulin level decreased to the basal level within about 2 h. On the other hand,

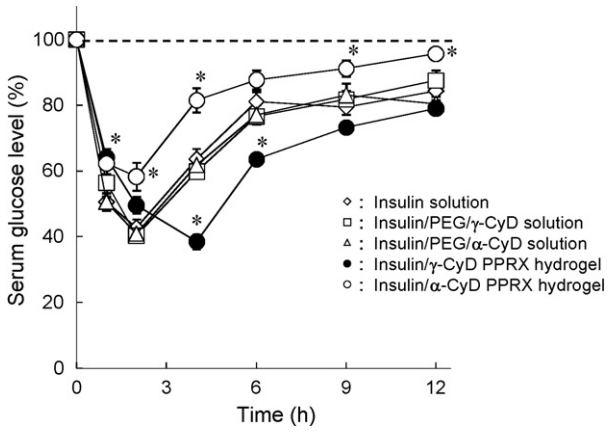

Fig. 8. Serum levels of glucose after subcutaneous administration of insulin, insulin/PEG/CyDs solution, insulin/α-CyD PPRX hydrogel and insulin/γ-CyD PPRX hydrogel to rats. Insulin/α-CyD PPRX hydrogel and insulin/γ-CyD PPRX hydrogel were diluted with 145 mg/mL α-CyD solution and 232 mg/mL γ-CyD solution, respectively. Each point represents the mean ± S.E. of ten experiments. *$p < 0.05$ versus insulin.

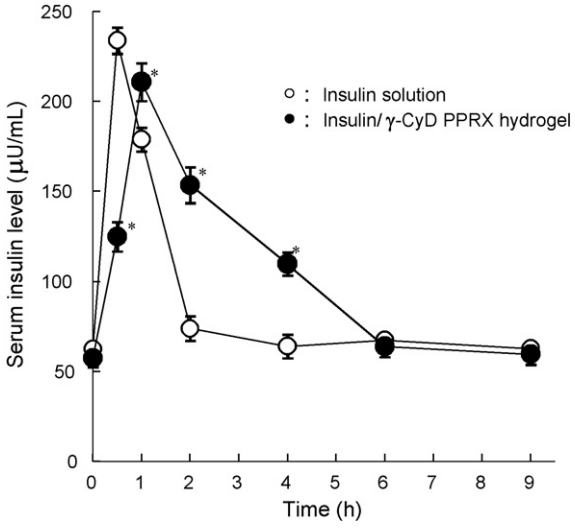

Fig. 9. Serum levels of insulin after subcutaneous administration of insulin and insulin/γ-CyD PPRX hydrogel to rats. Insulin/γ-CyD PPRX hydrogel was diluted with 232 mg/mL γ-CyD solution. Each point represents the mean ± S.E. of nine experiments. *$p < 0.05$ versus insulin.

Table 3
In vivo pharmacokinetic parameters of insulin and insulin/γ-CyD PPRX hydrogel systems.

Sample	T_{max} (h)[a]	C_{max} (μU/mL)[b]	AUC ((μU/mL)·h)[c]	MRT (h)[d]
Insulin	0.5	233.8 ± 7.3	768.0 ± 61.4	3.6 ± 0.03
Insulin/γ-CyD PPRX hydrogel	1*	210.8 ± 10.5	933.3 ± 59.5*	3.5 ± 0.04

Each value represents the mean ± S.E. of nine experiments.
[a] Time required to reach the maximum serum insulin level.
[b] Maximum serum insulin level.
[c] Area under the serum insulin level-time curve up to 9 h post-administration.
[d] Mean residence time in serum.
* $p < 0.05$ versus insulin.

insulin/γ-CyD PPRX hydrogel significantly sustained the serum insulin level and gradually recovered to the basal level after more than 6 h. The area under the serum insulin level-time curve of insulin/γ-CyD PPRX hydrogel was significantly increased, compared to that of insulin alone, suggesting that insulin/γ-CyD PPRX hydrogel can prolong the systemic circulation of insulin in vivo. These results suggest that insulin/γ-CyD PPRX hydrogel would be useful as a sustained release system for insulin after subcutaneous injection.

4. Conclusion

The growing importance of hydrogels in protein delivery applications has led to the development of many novel and promising preparation strategies. For the entrapment and encapsulation of labile bioactive substances, physically crosslinked hydrogels are of great interest especially once the gel formation occurs under mild condition in the absence of organic solvents. In this study, we demonstrated the formation of insulin/CyDs supramolecular hydrogels based on the physical crosslinking induced by the self-assembly of PEG threaded with α- and γ-CyD without any chemical crosslinking reagent involved. The release rate of insulin from supramolecular hydrogels can be controlled by adjusting the concentration of CyDs in the medium. These results suggest the potential use of γ-CyD PPRX hydrogel as an injectable sustained release system for insulin.

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