HHS Public Access

Author manuscript

Macromol Biosci. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:

Macromol Biosci. 2018 May ; 18(5): e1700372. doi:10.1002/mabi.201700372.

Glucose-Responsive Trehalose Hydrogel for Insulin Stabilization and Delivery[a]

Dr. Juneyoung Lee,
Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, USA. California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, USA

Jeong Hoon Ko,
Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, USA. California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, USA

Kathryn M. Mansfield,
Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, USA. California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, USA

Peter C. Nauka,
Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, USA. California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, USA

Prof. Erhan Bat, and
Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, USA. California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, USA. Department of Chemical Engineering, Middle East Technical University, Ankara 06800, Turkey

Prof. Heather D. Maynard
Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095-1569, USA. California NanoSystems Institute, University of California, Los Angeles, 570 Westwood Plaza, Los Angeles, California 90095, USA

Abstract

Effective delivery of therapeutic proteins is important for many biomedical applications. Yet, the stabilization of proteins during delivery and long-term storage remains a significant challenge. Herein, we report a trehalose-based hydrogel that stabilizes insulin to elevated temperatures prior to glucose-triggered release. The hydrogel is synthesized using a polymer with trehalose side chains and a phenylboronic acid end-functionalized 8-arm poly(ethylene glycol) (PEG). The

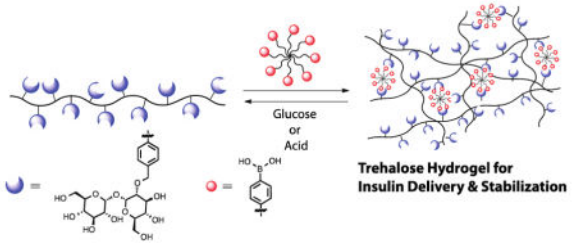
[a]Supporting Information is available online from the Wiley Online Library or from the author.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author

hydroxyls of the trehalose side chains form boronate ester linkages with the PEG-boronic acid cross-linker to yield hydrogels without any further modification of the original trehalose polymer. Dissolution of the hydrogel is triggered upon addition of glucose as a stronger binder to boronic acid (K_b = 2.57 M^{-1} vs. 0.48 M^{-1} for trehalose), allowing the insulin that was entrapped during gelation to be released in a glucose-responsive manner. Moreover, the trehalose hydrogel stabilizes the insulin as determined by immunobinding after heating up to 90 °C. After 30 min heating, 74% of insulin was detected by enzyme-linked immunosorbent assay (ELISA) in the presence of the trehalose hydrogel, whereas only 2% was detected without any additives.

Graphical abstract

A trehalose-boronic acid hydrogel is synthesized for glucose-responsive insulin delivery and stabilization. A trehalose glycopolymer is cross-linked with boronic acid end-functionalized PEG to encapsulate insulin. The protein is stabilized to heat when encapsulated in the hydrogel. Competitive displacement by glucose with the boronic acid facilitates glucose-responsive insulin release.



Keywords

biomaterials; drug delivery systems; hydrogels; proteins; stabilization

1. Introduction

Insulin was the first Food and Drug Administration (FDA)-approved recombinant protein drug, and is one of the most widely used treatment for diabetes.[1] However, one of the challenges associated with insulin therapy is the requirement of repeated injection or insertion of an insulin bolus after each meal in the case of the insulin pump, which may be problematic especially for children and active young adults.[2] To address these challenges, glucose-responsive insulin delivery systems have been proposed. Early works focused on the sugar binding capability of the lectin, concanavalin A (Con A).[3–5] While some of these systems demonstrated excellent glucose-responsive behavior,[4, 5] the toxicity[6] and potential denaturation[7] of Con A itself were pointed out as inherent problems with the materials. Another popular glucose-responsive material is the enzyme glucose oxidase,[8–10] but delivery systems based on glucose oxidase could also suffer in long term performance due to the instability of the enzyme.

More recently, phenylboronic acid that is non-toxic and durable has been widely used in materials for insulin release.[7, 11] Since boronic acid forms dynamic covalent complexes

with 1,2- or 1,3-diols,[12] its incorporation into hydrogels results in glucose-responsive materials. The two main mechanisms of insulin release reported from boronic acid hydrogels are swelling and competitive binding.[7, 11] The swelling mechanism is caused by the shift in the equilibrium of different boronic acid species toward the anionic tetrahedral form upon binding to diols such as those on sugars, which causes osmotic swelling of the hydrogels.[6] Alternatively, boronic acid-based polymers[13–15] can form a hydrogel upon complexation with diol-containing polymers in the presence of insulin, and later be competitively displaced by glucose to dissolve the hydrogel and release insulin.[16]

In addition to controlled release of insulin, the instability of the protein is an important issue that needs to be addressed. Exposure of insulin to changes in temperature during storage often leads to inactivation of the protein resulting in health complications for patients.[17] Instability also contributes to the medical costs of diabetes treatment from drug that is discarded and wasted.[18] While insulin has been modified to increase its half-life *in vivo* (by covalent attachment of a polymer)[19] and to prevent insulin hexamer formation (by mutation of the amino acid sequence),[20] few studies have reported stabilizing insulin to environmental heat exposure.[21, 22] The Peppas group has used nanospheres composed of poly(N-isopropylacrylamide) and poly(ethylene glycol) (PEG) to enhance thermal and mechanical stability of insulin,[21] but their system lacked a release mechanism. Sunamoto et al. have used cholesterol-bearing pullulan nanogels to stabilize insulin against heat and enzymatic degradation, and the nanogel released insulin when exposed to bovine serum albumin (BSA) levels by association of BSA with pullulan.[22] Although this system successfully stabilized insulin, it lacked glucose responsiveness, which is highly desirable in insulin delivery systems. To our knowledge, a hydrogel that is both glucose-responsive and insulin stabilizing has not yet been reported.

Our group has previously shown that trehalose glycopolymers are effective stabilizers for proteins, including insulin, against lyophilization and heat either as conjugates or as excipients.[23–27] We hypothesized that the trehalose glycopolymer, also named PolyProtek, could be used to entrap insulin by complexing with a boronic acid cross-linker and that the resulting hydrogel would also stabilize insulin against environmental stressors, while releasing the hormone upon competitive addition of insulin (Scheme 1). Herein, we describe results that demonstrate that the trehalose-boronic acid hydrogel can deliver insulin upon increase in glucose level, while also stabilizing the protein upon thermal stress.

2. Experimental Section

2.1. Materials

All chemicals were purchased from Sigma-Aldrich and Fisher Scientific and were used without purification unless noted otherwise. Recombinant human insulin was purchased from Sigma-Aldrich. Trehalose was purchased from The Healthy Essential Management Corporation (Houston, TX), and was azeotropically dried with ethanol and kept under vacuum until use. Azobisisobutyronitrile (AIBN) was recrystallized from acetone before use. 8-arm PEG amine was purchased from Jenkem Technology (Allen, TX). Human insulin ELISA kit was purchased from Mercodia (Uppsala, Sweden). Styrenyl ether trehalose monomer was prepared using our previously reported procedure.[23]

2.2. Analytical Techniques

NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer. Gel permeation chromatography (GPC) was conducted on a Shimadzu HPLC system equipped with a refractive index detector RID-10A and two Polymer Laboratories PLgel 5 μm mixed D columns (with guard column). Lithium bromide (0.1 M) in *N,N*-dimethylformamide (DMF) at 40 °C was used as the solvent (flow rate: 0.6 mL/min). Near-monodisperse poly(methyl methacrylate) standards (Polymer Laboratories) were employed for calibration. Infrared spectra were obtained with a Perkin-Elmer Spectrum One instrument equipped with a universal ATR accessory. Preparatory reverse phase HPLC was carried out on a Shimadzu HPLC system equipped with a UV detector using a Luna 5 μm C18 100 Å column (preparatory: 5 μm, 250 x 21.2 mm) with monitoring at λ = 215 nm and 254 nm. Isocratic solvent system (water:methanol = 50:50) was used as the mobile phase at a flow rate of 10 mL/min. Fluorescence measurement was made on a FlexStation II (Molecular Devices). UV-Vis absorbance was measured using a microplate reader ELx800 (BioTek Instruments, Winooski, VT). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis of FITC-labeled insulin was performed on a Bruker Ultraflex MALDI-TOF mass spectrometer in linear positive ion mode. FITC-labeled insulin (1 mg/mL) in Dulbecco's phosphate-buffered saline (D-PBS) was diluted 10-fold with deionized (Milli-Q filtered) water and mixed 1:1 with sinapinic acid (10 mg/mL) dissolved in 50% acetonitrile with 0.1 % trifluoroacetic acid on the MALDI target plate.

2.3. Determination of boronic acid binding constants

The method was adopted from the report by Deshayes *et al.*[28] Fluorescent boronic acid (2-naphthaleneboronic acid, λ_{ex} = 268 nm, λ_{em} = 344 nm) was dissolved at 335 μM concentration in Dulbecco's phosphate-buffered saline (D-PBS, pH 7.4) containing 2% DMSO, and then 150 μL of the boronic acid solution was mixed with equal volume of D-PBS solution containing trehalose or glucose. Fluorescence quenching was plotted as I_0 / I as a function of sugar concentration, where I_0 is the fluorescence in the absence of any sugar and I is the fluorescence in the presence of sugar. The intercept was fixed at 1, and the slope was taken to be the binding constant K_b according to the Stern-Volmer equation:

$$I_0/I = 1 + K_b[\text{quencher}] \quad (1)$$

2.4. Synthesis of trehalose polymer, poly(SET)

AIBN (5.28 mg, 3.22×10^{-2} mmol) and styrenyl ether trehalose monomer (634 mg, 1.38 mmol) were dissolved in a mixture of DMF (2.31 mL) and H_2O (4.61 mL). Oxygen was removed by three cycles of freeze-pump-thaw and polymerization was initiated at 75 °C. The polymerization was stopped after 8.5 h by immersing the reaction into liquid nitrogen. The polymer was purified by dialysis against H_2O (MWCO 3,500) resulting in a polymer with M_n = 7.0 kDa and $Đ$ = 1.28 (for hydrogel dissolution experiment) and M_n = 7.6 kDa and $Đ$ = 1.33 (for all other experiments). ^{1}H NMR (500 MHz in D_2O) δ: 7.01, 6.45, 5.05, 3.81, 3.71, 3.59, 3.48, 3.36, 1.50.

2.5. Synthesis of 8-arm PEG boronic acid

8-arm PEG amine (400 mg, 10 kDa, 4×10^{-2} mmol) and 4-formylphenylboronic acid (96 mg, 6.40×10^{-1} mmol) were dissolved in 2.8 mL of MeOH. $NaBH_3CN$ (37.7 mg, 6.00×10^{-1} mmol) was added and the reaction was stirred at 25 °C. After 5 days the reaction solution was purified by dialysis against MeOH for 3 days. The sample was lyophilized and the ^1H NMR spectrum was used to calculate the % modification of the amine end-groups of the PEG. For the aromatic peaks attached at the ends of 8-arm, there are 32 protons (8 arm x 4 aromatic protons per ring). For 10 kDa PEG, there are approximately 10,000 / 44 Da = 227 repeat units (i.e. "n" in the polymer structure is 227 / 8 = 28 per arm), and 227 x 4 protons per repeat unit = 908 protons. This calculation showed that approximately 100 % of the end-groups of the PEG were modified with the boronic acid. ^1H NMR (500 MHz in D_2O) δ: 7.75 (16 H), 7.41 (16 H), 3.69 (908 H). IR: δ = 3390, 2869, 1699, 1456, 1410, 1348, 1297, 1247, 1079, 1041, 986, 947, 839 cm^{-1}.

2.6. Hydrogel dissolution kinetics

The poly(SET) (500 mg/mL) and the PEG cross-linker (200 mg/mL) stock solutions were prepared in D-PBS, pH 7.4. The gels were prepared by adding 3 μL of the trehalose polymer stock solution and 20.5 μL of the PEG cross-linker stock solution and incubating at room temperature for 30 min. The gels were hydrated in D-PBS for 1 h, and then transferred to 5 mL D-PBS containing 0, 100, 500, 1000, or 2000 mg/dL glucose. At each time point, gels were weighed and then replaced into respective buffers.

2.7. FITC labeling of insulin

Insulin was labeled with fluorescein isothiocyanate isomer I (FITC) by dissolving insulin (0.65 mg, 0.112 μmol) and FITC (3.48 mg, 8.94 μmol) in 0.33 mL of 1 M sodium bicarbonate buffer, pH 8.3. The mixture was stirred for two hours, and free FITC was removed by repeated centrifugation through a membrane using Centriprep™ tubes with molecular weight cut-off (MWCO) of 3 kDa. Typical degree of labeling was approximately 0.7 FITC per insulin as determined by UV absorbance.[29] The MALDI-TOF spectrum (Figure S2) confirmed labeling of insulin with 1 or 2 FITC molecules, which is in accordance with the expected reactivity of the three reactive amine groups on insulin (GlyA1, PheB1, and LysB29), with GlyA1 and LysB29 being much more reactive than PheB1.[25]

2.8. FITC-labeled insulin release from trehalose hydrogel

FITC-labeled insulin (13.22 mg/mL in D-PBS, pH 7.4 or pH 8) was added to the trehalose polymer to make a polymer concentration of 500 mg/mL. The PEG cross-linker was dissolved in D-PBS at 200 mg/mL concentration. Next, 1 μL of the trehalose polymer and FITC-labeled insulin stock solution and 6.84 μL of the PEG cross-linker stock solution were added to an Eppendorf Lo-Bind® centrifuge tube. The tube was agitated on a ThermoShaker (Allsheng Instruments, China) at 1,500 rpm at 21 °C for 1 h. The gels were transferred into a 24-well plate filled with 1 mL D-PBS and left to hydrate for 30 min. Next, the gels were transferred to a 96-well plate that had been blocked with 1% wt/vol BSA in D-PBS to prevent protein adsorption and filled with 0.3 mL of D-PBS containing 0, 500, or 1000

mg/dL glucose. At each time point, all the solution was aliquoted and the wells containing the gels were immediately refilled with 0.3 mL of the same buffer. After the last time point, the wells were treated with 0.3 mL of D-PBS containing 10000 mg/dL glucose and incubated at 37 °C for 5 min to completely dissolve the gels. All the solution was then transferred for measurement, and fluorescence of the time point aliquots and the residual insulin solutions recovered after gel dissolution was measured.

2.9. Trehalose hydrogel heating assay

A stock insulin solution was prepared by first dissolving insulin in D-PBS, pH 7.4 at 100 mg/dL concentration, and then concentrated by centrifugation through a membrane using Centriprep™ tubes with molecular weight cut-off (MWCO) of 3 kDa. The protein concentration was quantified by UV absorbance at 280 nm, and the solution was diluted to 3.93 mg/mL such that the final insulin concentration in the samples was 0.5 mg/mL. Trehalose polymer stock solution was prepared by dissolving poly(SET) in the insulin stock solution at a 500 mg/mL concentration. The PEG cross-linker was dissolved in D-PBS at 200 mg/mL concentration. The gels were prepared by adding 1 μL of insulin or trehalose polymer stock solution and 6.84 μL of PEG cross-linker stock solution or D-PBS to an Eppendorf Lo-Bind® centrifuge tube, and agitating the tube on a ThermoShaker at 1,500 rpm at 21 °C for 1 h to aid in mixing. The samples were heated at 90 °C for 30 min and the controls were kept at 4 °C. All samples and controls were treated with 1 mL of 10000 mg/dL glucose in order to dissolve the hydrogel. The amount of insulin was assayed by ELISA, which was conducted according to manufacturer's instructions. Briefly, 25 μL of the diluted samples were added to the wells pre-coated with the capture antibody. Buffer containing detection antibody was added (100 μL), and the plate was incubated on a rocker at room temperature for 1 h. To prevent residual boronic acid binding to the sugar moieties on horseradish peroxidase used for ELISA,[30, 31] the wells were washed with 350 μL of deionized water acidified with HCl (pH = 3.5) five times after the incubation, and then six times with 350 μL of the wash buffer. These additional washing steps do not affect the ELISA results as confirmed by the controls. 3,3′,5,5′-Tetramethylbenzidine (TMB) solution was added (200 μL), and the plate was incubated at room temperature for 15 min before the addition of 50 μL stop solution (1 N H_2SO_4). The amount of insulin detected was quantified by absorbance at 450 nm relative to the standards supplied by the manufacturer.

2.10. Statistical analysis

One-tailed Student's t-test assuming unequal sample variance was used to test the difference between experimental groups. Results were considered significantly different if $p < 0.05$.

3. Results and Discussion

We designed the three-component glucose-responsive insulin delivery system consisting of trehalose polymer (poly(styrenyl ether trehalose) or poly(SET)), multivalent boronic acid cross-linker (8-arm PEG boronic acid), and insulin (Scheme 1). When trehalose and boronic acid polymers are mixed in the presence of insulin, the diols in trehalose polymer should form the tetrahedral boronate ester with boronic acid moieties and physically entrap insulin within the hydrogel pores. We anticipated that when glucose was added, the 1,2 diols in

glucose would competitively displace the 1,3 diols of trehalose to dissolve the hydrogel, leading to insulin release.

Ideally, glucose as the competitive displacer of trehalose polymer should have higher binding to boronic acid in order for the system to exhibit high sensitivity to glucose. Some studies have found no binding affinity between trehalose and boronic acid, limiting its usefulness for applications such as sugar sensing.[32] Yet, other studies have demonstrated weak binding to trehalose.[33] Thus to both confirm trehalose-boronic acid interaction and compare glucose and trehalose binding affinities, we measured the binding constants using a fluorescence-based method (Figure 1).[28] Fluorescence of the boronic acid probe was quenched by trehalose in a concentration-dependent manner at neutral pH, showing that 1,3 diol does indeed bind to boronic acid. Moreover, glucose had 5.4 times higher binding than trehalose (2.57 versus 0.48 M^{-1}), providing support that competitive displacement by glucose was possible. Moreover, we expected the multivalent effect of the polymer to help facilitate gel formation due to an increase in local concentration of diols available to form cross-links.

With trehalose binding to boronic acid confirmed to be non-zero but weaker than glucose, we then prepared non-covalent hydrogels for study. Even though individually at neutral pH, the binding of the boronic acid to the trehalose will be weak, because of the multivalency of many different trehalose units (average number of 17 per chain for pSET molecular weight of 7.6 kDa) the binding can still occur to form a gel. For the materials, a boronic acid cross-linker was synthesized through reductive amination, using 4-formylphenylboronic acid and 8-arm PEG amine as starting materials (Scheme S1). Complete modification of the amine end-groups with phenylboronic acid was confirmed by ^{1}H NMR spectroscopy (Figure S1). Next, the trehalose hydrogel was prepared by mixing the 8-arm PEG boronic acid with Poly(SET) at a 1:1 molar ratio of boronic acid to trehalose units (Scheme 1) in Dulbecco's phosphate-buffered saline (D-PBS). The gelation occurred rapidly after mixing the solutions of the two components (see Figure 2 for photos of the hydrogels). It was viscous within a minute and forms the gel within 5 minutes. Thus we envision that the materials may be injectable as the hydrogel is fast-forming after mixing.

The prepared poly(SET)-boronic ester hydrogel was then tested for glucose responsiveness. Trehalose-boronic acid binding has been observed for multivalent boronic acid-DNA conjugates,[34] and the association constant of boric acid with trehalose was measured to be smaller than glucose,[33] and this was confirmed through our fluorescence assay. To test this in the hydrogel system, the kinetics of hydrogel dissolution were monitored by measuring hydrogel weight upon addition of glucose. As shown in Figure 3(a), when the hydrogels were placed into the buffer (D-PBS, pH 7.4) containing glucose, the rate of percent weight loss was significantly faster with increasing glucose concentration. The weights of the hydrogels immersed in 1000 and 2000 mg/dL glucose solutions could not be measured after 10 minutes because the hydrogels had completely dissolved and were undetectable in the solution, while hydrogels in 100 mg/dL and 500 mg/dL glucose solutions were still intact after 60 min. Approximately 34% weight loss was observed after immersing the gel in D-PBS without any glucose for 60 minutes. Since the boronate ester bond is in dynamic equilibrium and the bond to trehalose is weak, the trehalose polymer may slowly diffuse out

from the hydrogel surface even in the absence of glucose. Yet with addition of glucose, the weight loss was remarkably accelerated, demonstrating the glucose-responsiveness of the gels.

To test insulin release upon addition of glucose, the poly(SET) boronic acid hydrogels were prepared in the presence of FITC-labeled insulin (Figure 2b). 8-Arm PEG boronic acid was dissolved in a buffer containing FITC-labeled insulin and mixed with the poly(SET) to prepare hydrogels, and these were added into D-PBS containing 0, 100, 200, 500, and 1000 mg/dL glucose. At first the release at pH 8.0 was tested since boronic acid-diol binding is stronger at basic pH.[6] Aliquots were taken from the solutions at each time point and released insulin was quantified (Figure S3). As with the gel dissolution experiment, glucose responsive behavior was observed, with slower release at lower glucose concentrations (100, 200, and 500 mg/mL) and faster release at high glucose concentrations (1000 mg/mL). We also conducted the release experiment at pH 7.4 to characterize the glucose-responsive insulin release at a physiological pH (Figure 3b). We utilized 0, 500, and 1000 mg/dL glucose levels since the pH 8 data had demonstrated no statistical differences between 100, 200 and 500 mg/dL. After one hour, the hydrogel in the 1000 mg/dL glucose solution was completely dissolved to yield 99 ± 1 % insulin release, while over the same time period 80 ± 9 % and 49 ± 12 % insulin were released in 500 mg/dL and 0 mg/dL glucose solution, respectively. The gel could be completely dissolved with 0.1 M HCl (pH 1); as expected the boronic acid-diol interaction is disrupted at acidic pH.[28] Together, the data shows that the hydrogel was able to release insulin in a glucose-dependent manner, and the system may be fine-tuned in the future to exhibit a more sensitive release profile in accordance with the narrow therapeutic window. For example, the release in more basic buffer (pH 8.0) was slower than at neutral pH suggesting that the pKa of boronic acid may be tailored as desired for more rapid or delayed insulin delivery. This has been exploited in other systems.[6, 14, 35] Additionally, improved stability of the ester using B-O dative bond formation may be utilized to tune the insulin delivery at neutral pH.[36] Another possible approach to increase the binding affinity and thus decrease the insulin release at low glucose levels is to incorporate a monomer with a cis-1,2 diol into the trehalose polymer chains.

Next, we tested the ability of the trehalose hydrogel to stabilize insulin against heating. Insulin solutions were separately prepared without any additive, with poly(SET), with 8-arm PEG boronic acid, or with the trehalose hydrogel. The samples were heated for 30 min at 90 °C to accelerate heat-induced degradation and then tested with insulin ELISA to confirm the structural integrity of insulin. A control group with insulin and the trehalose hydrogel stored at 4 °C demonstrated that the hydrogel did not affect the ELISA results (second entry compared to the first entry, Figure 4). It is interesting to note that the hydrogel lost its water content under these conditions but could be rehydrated following exposure to heat (Figure S4), indicating that the network remained intact or readily reformed. We have observed that trehalose polymers stabilize proteins to both heat and removal of water (lyophilization).[23] We hypothesize that the presence of the trehalose network stabilizes insulin in a similar manner even if the hydrogel is dehydrated during the heating process.

The data shows that the glucose-responsive trehalose hydrogel is effective at stabilizing insulin against heating stress (Figure 4). Insulin without any additive underwent degradation

and no longer bound to the antibody upon heating, showing less than 2% signal by ELISA. Significantly more insulin was detected in the presence of additives. Poly(SET) remarkably stabilized insulin and 63 ± 15 % of the original protein was detected after heating to 90 °C for 30 min. Insulin was also partially stabilized in the presence of the 8-arm PEG boronic acid alone (39 ± 13%). The literature is divided on the effect of PEG on protein stability; it has been suggested that PEG may accelerate protein denaturation at higher temperatures due to the interaction of hydrophobic PEG with the denatured state of protein.[37, 38] However, the specific architecture of PEG polymer may dictate whether PEG stabilizes or destabilizes proteins. For example, Amirgoulova et al. have reported that linear PEG interacts with the denatured state of a protein to favor unfolding, and they therefore used star-shaped PEG instead for their surface coating applications.[39] Importantly, the combination of both poly(SET) and branched PEG as a hydrogel resulted in 74 ± 22% stabilization, significantly better than the 8-arm PEG boronic acid ($p < 0.01$) and similar to poly(SET) alone. These results suggest that even though the poly(SET) is partially bound to the 8-arm PEG boronic acid in the gel, the stabilizing properties of the polymer are maintained.

4. Conclusions

In summary, we have synthesized a glucose-responsive hydrogel based on a trehalose glycopolymer for insulin delivery and stabilization against heat. The results demonstrate that hydrogels can be readily prepared from trehalose polymers and boronic acid cross linkers. The gelation occurred under physiological conditions, and the resulting hydrogel was capable of releasing insulin in a glucose-responsive manner. The addition of glucose led to breaking of the boronate ester bond between the trehalose polymer and the boronic acid cross-linker through competitive displacement by glucose, which has 5.4 times higher binding affinity to boronic acid than trehalose. As expected, higher glucose concentration in the buffer increased the rate of dissolution of the hydrogel and resulted in faster release of loaded insulin. Additionally, the trehalose hydrogel can effectively protect insulin against heat stress. Since most of the protein drugs must be stored under regulated temperature to maintain their activities, trehalose hydrogels in general may be used to enhance the quality of life of patients by not requiring specialized refrigeration, and this is being tested. Also, as boronic acid has been used to create pH-responsive materials,[35] the trehalose boronic-acid hydrogels may have potential applications for delivery of a wider range of protein therapeutics. For example, this has been exploited to release anti-cancer drugs such as therapeutic antibodies at acidic extracellular pH near tumors.[40]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Science Foundation (CHE-1112550 and CHE-1507735). J.L. and K.M.M. thank the NIH Biotechnology Training Fellowship (T32 GM067555) for funding. E.B. thanks The Netherlands Organization for Scientific Re-search and Marie Curie Cofund Action for the financial support (Rubicon Grant 680-50-1101).

References

1. Brown LR. Expert Opin Drug Delivery. 2005; 2:29.
2. Burdick J, Chase HP, Slover RH, Knievel K, Scrimgeour L, Maniatis AK, Klingensmith GJ. Pediatrics. 2004; 113:e221. [PubMed: 14993580]
3. Brownlee M, Cerami A. Science. 1979; 206:1190. [PubMed: 505005]
4. Kim JJ, Park K. J Controlled Release. 2001; 77:39.
5. Yin RX, Wang KM, Du S, Chen L, Nie J, Zhang WJ. Carbohydr Polym. 2014; 103:369. [PubMed: 24528742]
6. Matsumoto A, Ishii T, Nishida J, Matsumoto H, Kataoka K, Miyahara Y. Angew Chem, Int Ed Engl. 2012; 51:2124. [PubMed: 22162189]
7. Wu Q, Wang L, Yu H, Wang J, Chen Z. Chem Rev. 2011; 111:7855. [PubMed: 21902252]
8. Gough DA, Armour JC. Diabetes. 1995; 44:1005. [PubMed: 7657021]
9. He C, Liu J, Xie L, Zhang Q, Li C, Gui D, Zhang G, Wu C. Langmuir. 2009; 25:13456. [PubMed: 19778049]
10. Renard E. Curr Opin Pharmacol. 2002; 2:708. [PubMed: 12482735]
11. Brooks WLA, Sumerlin BS. Chem Rev. 2016; 116:1375. [PubMed: 26367140]
12. Cambre JN, Sumerlin BS. Polymer. 2011; 52:4631.
13. Bapat AP, Roy D, Ray JG, Savin DA, Sumerlin BS. J Am Chem Soc. 2011; 133:19832. [PubMed: 22103352]
14. Yesilyurt V, Webber MJ, Appel EA, Godwin C, Langer RS, Anderson DG. Adv Mater. 2016; 28:86. [PubMed: 26540021]
15. Dong Y, Wang W, Veiseh O, Appel EA, Xue K, Webber MJ, Tang BC, Yang X, Weir GC, Langer RS. Langmuir. 2016; 32:8743. [PubMed: 27455412]
16. Wang YCZ, Ma L, Shi C, Shen T, Song J. RSC Adv. 2014
17. Pryce R. BMJ. 2009; 338
18. Weiss RC, van Amerongen D, Bazalo G, Aagren M, Bouchard JR. Managed Care. 2011; 20:42.
19. Hinds KD, Kim SW. Adv Drug Delivery Rev. 2002; 54:505.
20. Heise T, Nosek L, Spitzer H, Heinemann L, Niemoller E, Frick AD, Becker RH. Diabetes, Obes Metab. 2007; 9:746. [PubMed: 17593235]
21. Leobandung W, Ichikawa H, Fukumori Y, Peppas NA. J Controlled Release. 2002; 80:357.
22. Akiyoshi K, Kobayashi S, Shichibe S, Mix D, Baudys M, Kim SW, Sunamoto J. J Controlled Release. 1998; 54:313.
23. Lee J, Lin EW, Lau UY, Hedrick JL, Bat E, Maynard HD. Biomacromolecules. 2013; 14:2561. [PubMed: 23777473]
24. Mancini RJ, Lee J, Maynard HD. J Am Chem Soc. 2012; 134:8474. [PubMed: 22519420]
25. Liu Y, Lee J, Mansfield KM, Ko JH, Sallam S, Wesdemiotis C, Maynard HD. Bioconjugate Chem. 2017; 28:836.
26. Messina MS, Ko JH, Yang Z, Strouse MJ, Houk KN, Maynard HD. Polym Chem. 2017; 8:4781.
27. Pelegri-O'Day EM, Paluck SJ, Maynard HD. J Am Chem Soc. 2017; 139:1145. [PubMed: 28079370]
28. Deshayes S, Cabral H, Ishii T, Miura Y, Kobayashi S, Yamashita T, Matsumoto A, Miyahara Y, Nishiyama N, Kataoka K. J Am Chem Soc. 2013; 135:15501. [PubMed: 24028269]
29. Schreiber AB, Hoebeke J, Bergman Y, Haimovich J, Strosberg AD. J Immunol. 1978; 121:19. [PubMed: 670698]
30. Ye J, Chen Y, Liu Z. Angew Chem, Int Ed. 2014; 53:10386.
31. Zhang W, Liu W, Li P, Xiao H, Wang H, Tang B. Angew Chem, Int Ed. 2014; 53:12489.
32. James TD, Sandanayake KRAS, Shinkai S. Angew Chem Int Edit. 1996; 35:1910.
33. Van den Berg R, Peters JA, Van Bekkum H. Carbohydr Res. 1994; 253:1. [PubMed: 8156542]
34. Hargrove AE, Ellington AD, Anslyn EV, Sessler JL. Bioconjug Chem. 2011; 22:388. [PubMed: 21299200]

35. Roy D, Cambre JN, Sumerlin BS. Chem Commun. 2009; 2106
36. Deng CC, Brooks WLA, Abboud KA, Sumerlin BS. ACS Macro Lett. 2015; 4:220.
37. Lee LLY, Lee JC. Biochemistry. 1987; 26:7813. [PubMed: 3427106]
38. Senske M, Tork L, Born B, Havenith M, Herrmann C, Ebbinghaus S. J Am Chem Soc. 2014; 136:9036. [PubMed: 24888734]
39. Amirgoulova EV, Groll J, Heyes CD, Ameringer T, Rocker C, Moller M, Nienhaus GU. ChemPhysChem. 2004; 5:552. [PubMed: 15139230]
40. Lee ES, Gao Z, Bae YH. J Controlled Release. 2008; 132:164.

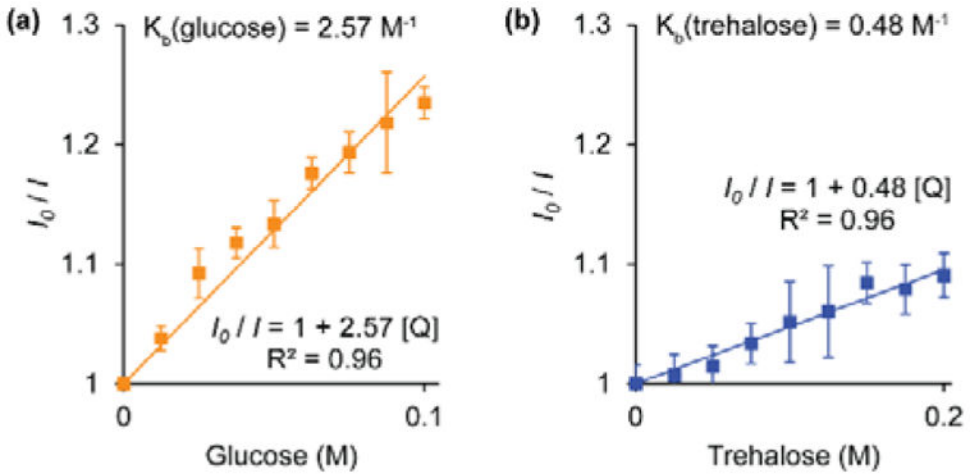


Figure 1.

Relative fluorescence of boronic acid at pH 7.4 as a function of (a) glucose (n = 3) and (b) trehalose concentration (n = 5 or 6).

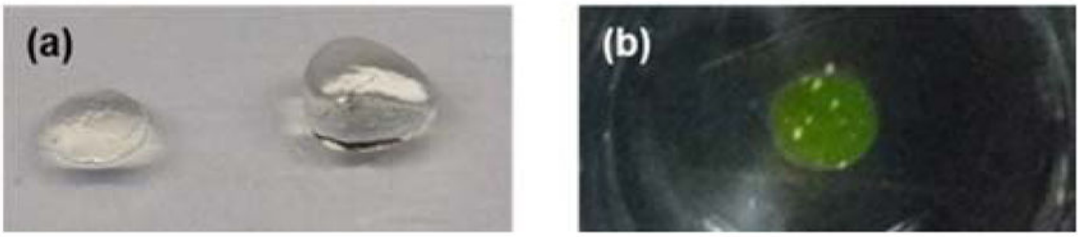


Figure 2.
(a) Photograph of the formed trehalose-boronic acid hydrogel. (b) Trehalose-boronic acid hydrogel loaded with FITC-labeled insulin in pH 7.4 D-PBS.

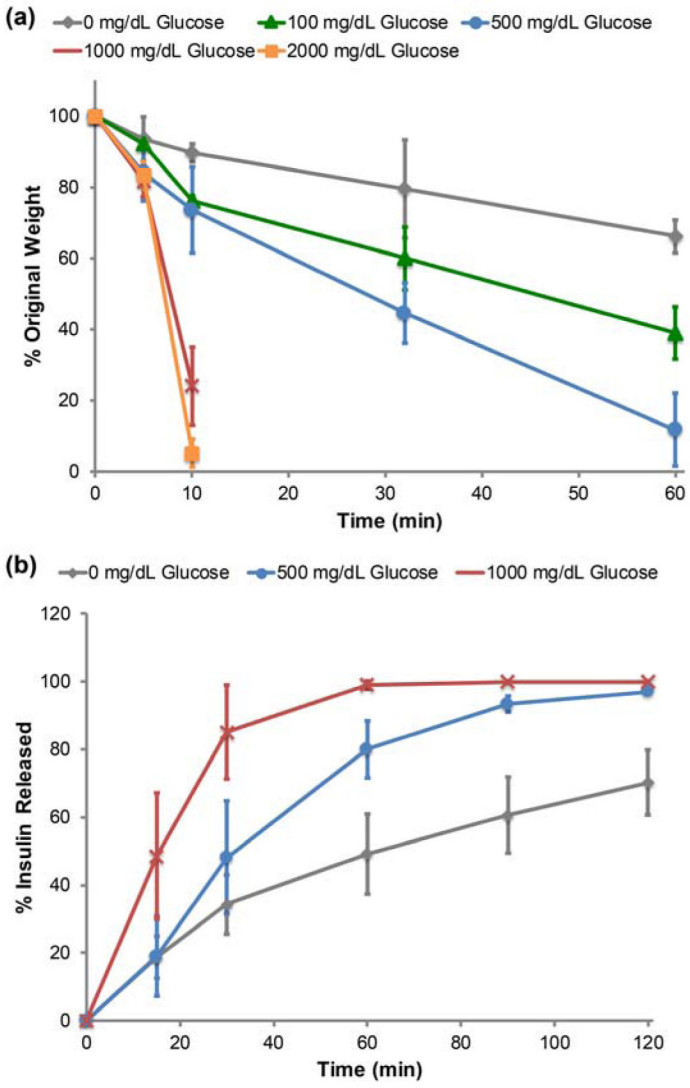


Figure 3.

(a) Dissolution kinetics of poly(SET)-boronic acid hydrogels after immersing into D-PBS, pH 7.4, containing 0, 100, 500, 1000, and 2000 mg/dL glucose (n=3 per group). (b) Insulin released in D-PBS, pH 7.4, containing 0, 500, and 1000 mg/dL glucose (n=6 per group).

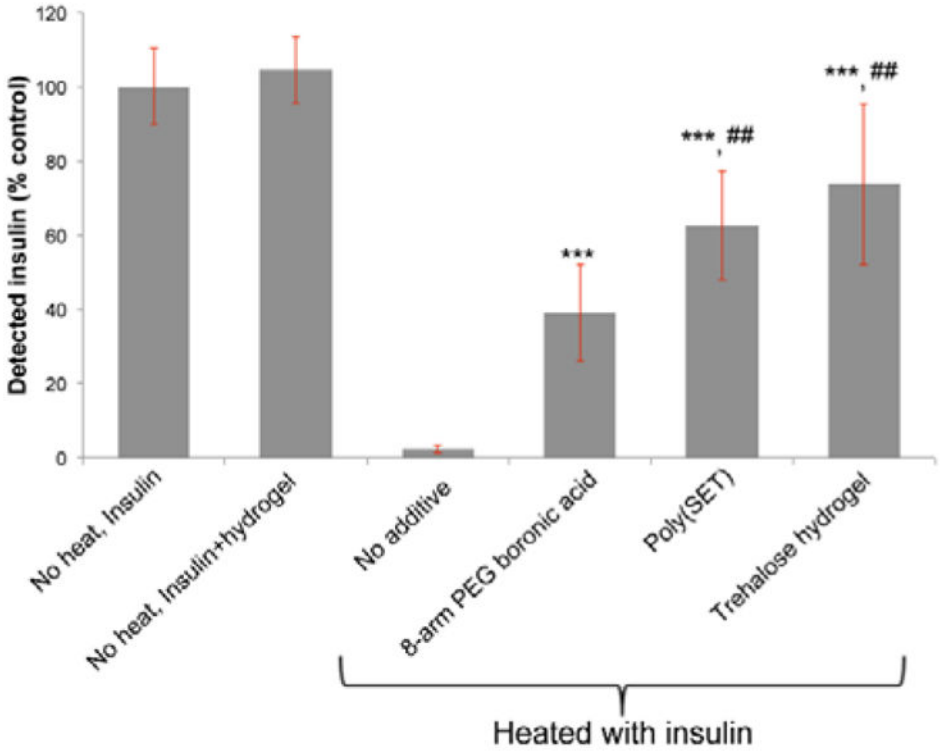
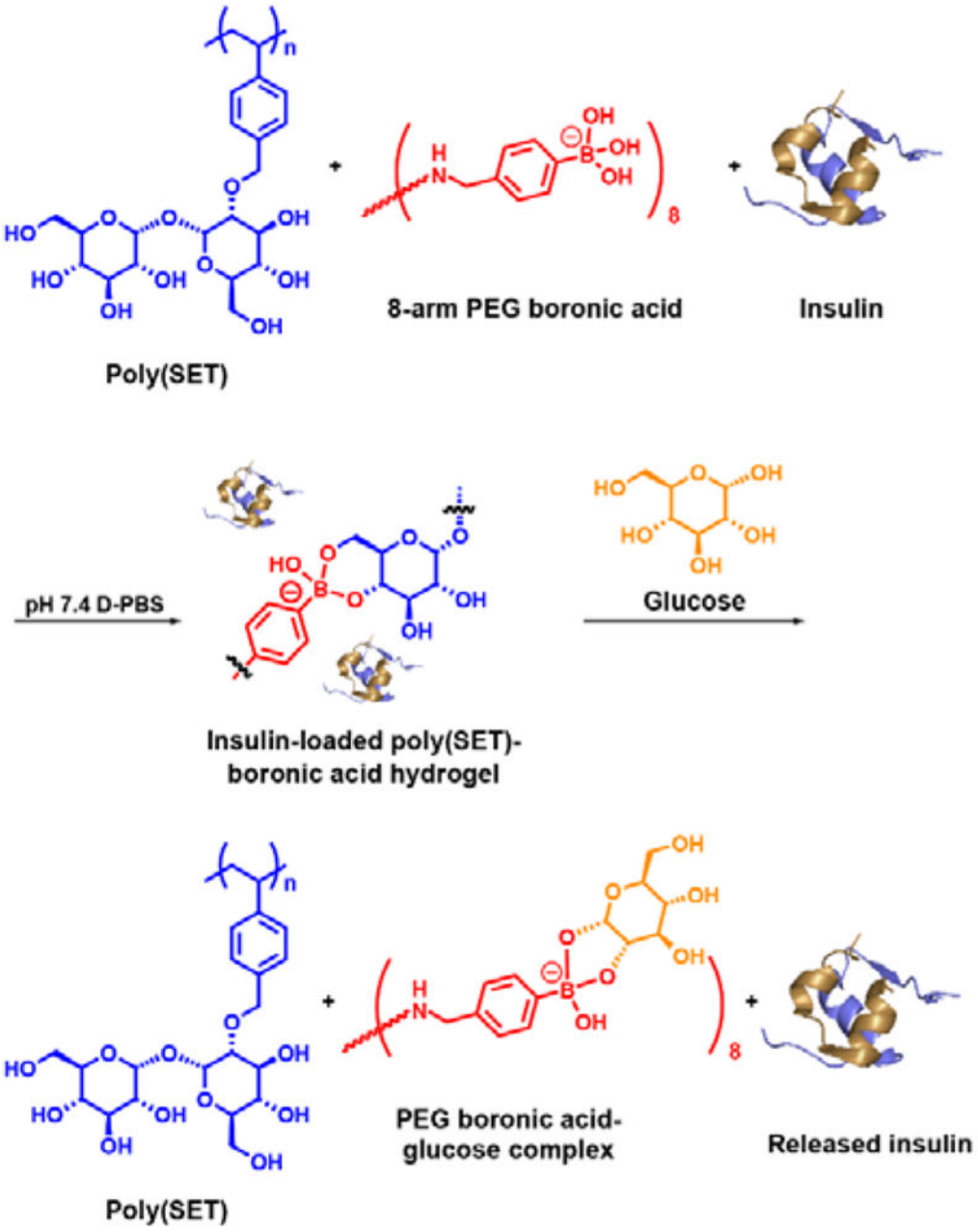


Figure 4.

ELISA results of insulin (no heat control), insulin with hydrogel (no heat control), insulin with no additive (heated), insulin with 8-arm PEG boronic acid (heated), insulin with trehalose polymer (heated), and insulin with hydrogel (heated). Heating condition was 90 °C for 30 min. *** is $p < 0.001$ relative to no additive, ## is $p < 0.01$ relative to 8-arm PEG boronic acid (n=6).



Scheme 1.

Design for insulin delivery using trehalose-boronic acid hydrogel (insulin PDB ID: 4INS).