Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 3597–3606
Biomaterials
www.elsevier.com/locate/biomaterials

Synthesis, bioactivity and specificity of glucagon-like peptide-1 (7–37)/polymer conjugate to isolated rat islets

Sungwon Kim[a,b], Sung Wan Kim[a], You Han Bae[a,*]

[a]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, 421 Wakara Way Suite 318, Salt Lake City, UT 84108, USA
[b]Department of Materials Science and Engineering, Center for Biomaterials and Bioengineering, Gwangju Institute of Science and Technology, 1 Oryong-dong, Buk-gu, Gwangju 500-712, Republic of Korea

Received 26 April 2004; accepted 8 September 2004
Available online 10 November 2004

Abstract

In order to increase the functionality of islets encapsulated in a biohybrid artificial pancreas (BAP), it was proposed that co-encapsulation with insulinotropic agents would improve insulin secretion from islets. To prevent agents from leaking out, conjugation with high-molecular-weight polymers was inevitable. In this study, synthetic glucagon-like peptide-1 (GLP-1) (7–37) was conjugated to a water-soluble polymer, poly(*N*-vinyl-2-pyrroridone-co-acrylic acid) (5 mol% acrylic acid, M_w 445 kDa), via poly(ethylene glycol, M_w 3.4 kDa) spacer. The chemical conjugation was confirmed by reverse phase-HPLC and the GLP-1 content in the GLP-1/polymer conjugate (VAPG) was determined by UV spectrophotometry at 280 nm (ca. 29 wt/wt%). In a static insulin secretion test, the VAPG increased insulin secretion up to 200% over a control (no stimulation) at high glucose levels, although the insulinotropic activity of VAPG was slightly lower than that of native GLP-1. The bioactivity of VAPG was prolonged for at least 2 weeks, which was examined by co-encapsulation of the conjugate into islet microcapsules. Dose–response curve revealed that the half-maximal effective dose (ED_{50}) of VAPG was about 55 nM (25 nM for native GLP-1). By N-terminal analysis using aminopeptidase and RP-HPLC, it was confirmed that the lowered bioactivity of VAPG stemmed from the polymer conjugation to N-terminal histidine moieties, which actively participate in binding to GLP-1 receptors, resulting in only 16% of N-terminal histidine remaining intact after the conjugation reaction. Finally, the specific interaction of the VAPG with isolated rat islets was investigated. Total cellular cyclic AMP levels were measured and confocal microscopy was conducted using GLP-1 and VAPG labeled with fluorescent probes. It was found that VAPG effectively increased the cAMP level in islet cells in a glucose concentration-dependent manner. Moreover, the confocal microscopy study showed that the binding of VAPG occurs at the same location where GLP-1 binds but with less affinity than that of native GLP-1. In summary, a GLP-1/polymer conjugate was synthesized for the first time, and its bioactivity was examined, which must result from its specific interaction with isolated islets.

Keywords: Glucagon-like peptide-1 (GLP-1); Polymer; Insulin secretion; Islets of Langerhans; Biohybrid artificial pancreas

1. Introduction

The biohybrid artificial pancreas (BAP) is an artificial organ designed to treat type 1 diabetes that results from irreversible destruction of insulin-secreting pancreatic β-cells [1,2]. Even though numerous investigations have been accomplished to realize the application of a BAP in a clinical setting, significant obstacles still remain including the size of implant, the number of islets required for normoglycemia and the deteriorated insulin-secreting function of islets during isolation and encapsulation procedures [3]. To subjugate these problems, we have proposed that if insulinotropic agents are incorporated into the BAP, the number of islets needed to normalize blood glucose of a diabetic patient can be reduced by improving the insulin secretion ability

*Corresponding author. Tel.: +1 801 585 1518; fax: +1 801 585 3614.
E-mail address: you.bae@m.cc.utah.edu (Y.H. Bae).

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.09.045

of encapsulated islets, which has been reviewed by Hou and Bae [1]. To realize this hypothesis, our group has conducted several studies with sulfonylurea, a type of hypoglycemic drug, and glucagon-like peptide-1 (GLP-1) [4–7]. When their functions are expired, the entire contents of a BAP can be removed and refreshed, allowing the restoration of normoglycemia.

GLP-1 is one of the incretin hormones, which stimulates insulin secretion depending on the blood glucose concentration in the body, and responds effectively to higher glucose levels. The major portion of GLP-1 in plasma is produced by intestinal L-cells and processed from proglucagon by specific enzymatic cleavage [8]. While it has been known that the distribution of GLP-1 is limited to pancreatic α-cells, central nervous system and intestinal L-cells, GLP-1 receptors have a wide tissue distribution including pancreas, brain, hypothalamus, intestine, stomach, kidney, heart and lung [9]. The key role of GLP-1 in energy homeostasis is to regulate blood glucose levels by stimulating pancreatic β-cells after meal ingestion.

Previously, we investigated the short-term bioactivity of GLP-1/Zn^{2+} crystal using islet macrocapsules [7]. In this study, GLP-1 was conjugated to a water-soluble polymer to examine the feasibility of creating a polymeric insulinotropic agent which would be incorporated into a BAP and expected to enhance the functionality of islets. The reason for conjugating a high-molecular-weight polymer to GLP-1 was to prevent the peptide from leaking out of a BAP device rather than to improve its biological activity as a therapeutic drug by elongating the circulation time. After confirming the chemical conjugation between GLP-1 and the polymer, the bioactivity and specificity of the conjugate were tested on isolated rat islets. We believe this is not only the first trial to conjugate GLP-1 to a macromolecule but we also will gain a better understanding of GLP-1 bioactivity and biochemistry.

2. Materials and methods

2.1. Materials

Synthetic GLP-1 (7–37) was a kind gift from Dr. M. Baudyš (MacroMed Inc., Sandy, UT). A heterobifunctional ω-amino-α-carboxyl poly(ethylene glycol) (M_w 3400; HCl•NH_2-PEG_{3400}-COOH) was purchased from Shearwater Polymers (Huntsville, AL). *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetonitrile (AcN), dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), triethylamine (TEA), L-histidine monohydrochloride monohydrate and anhydrous diethyl ether were purchased from Aldrich Chemical Co. (St. Louis, MO). Spectra/Por®6 dialysis tubing (MWCO 15,000) was purchased from Spectrum Laboratories Inc., (Rancho Dominguez, CA). RPMI-1640 medium, collagenase type V, Ficoll 400-DL, glucose, sodium bicarbonate ($NaHCO_3$), 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), glutaldehyde, aminopeptidase M and formaldehyde were obtained from SIGMA Chemicals Co. (St. Louis, MO). Hank's balanced salt solution (HBSS), penicillin/streptomycin antibiotics, fetal bovine serum (FBS) and sodium pyruvate were purchased from Invitrogen Corporation (GibcoBRL, Grand Island, NY). All chemical reagents and organic solvents used were at least ACS grade.

2.2. Synthesis PEG-grafted poly(*N*-vinyl-2-pyrrolidone-co-acrylic acid) (VAP)

Fig. 1 illustrates the synthetic scheme of GLP-1/polymer conjugate. The carboxyl groups (46.9 μmol) of 1.5 g poly(*N*-vinyl-2-pyrrolidone-co-acrylic acid) (VA) were activated by 14.4 mg of DCC (70.0 μmol), 5.75 mg of NHS (50.0 μmol) and dry 0.1 mL TEA in 100 mL anhydrous DMF. The VA was the same material as characterized in our previous report [4]; M_w 445 kDa by light scattering method, 312.5 μmol acrylic acid/g VA polymer. The reaction was carried out for 48 h under N_2 atmosphere followed by precipitation twice against excess anhydrous diethyl ether (1.2 L). The product was dried and stored in vacuo with P_2O_5 for further reaction (Yield = 1.4 g). The solution of 130 mg heterobifunctional PEG (HCl•NH_2-PEG_{3400}-COOH, 38.3 μmol) pre-dissolved in 10 mL dry DMF with 0.1 mL TEA an hour before use was poured into the activated VA (100.1 mg, 29.4 μmol acid content) solution in 50 mL dried DMF together with 0.1 mL TEA. After 48 h reaction under N_2 atmosphere, the product was collected by precipitation twice against excess diethyl ether. Then, the VA solution in 20 mL H_2O was dialyzed against excess distilled water for 5 days (MWCO 15 kDa). Retrieved solution was lyophilized and stored in vacuo till further experiment (Yield = 162.1 mg). By assuming that 100% coupling took place between PEG and VA, the acid content of VA (312.5 μmol) was adopted as the number of carboxyl ends of PEG in VAP without further characterization because the GLP-1 content was expected to be acquired by another method described later.

2.3. Synthesis of GLP-1/VAP conjugate (VAPG)

First, the carboxylic end groups of PEG (9.2 μmol) of VAP (61.0 mg) were converted to active forms by reaction in 20 mL anhydrous DMSO with 1.2 mg NHS (10.0 μmol), 2.5 mg DCC (12.0 μmol), and 10 μL dry TEA. After 24 h reaction under N_2 atmosphere, 40.0 mg GLP-1 (12.1 μmol) dissolved in 15 mL anhydrous DMSO with 0.1 mL TEA was added. Because GLP-1