Journal of Controlled Release 241 (2016) 25–33

Contents lists available at ScienceDirect

Journal of Controlled Release

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

The molecular basis for the prolonged blood circulation of lipidated incretin peptides: Peptide oligomerization or binding to serum albumin?

Ying Wang [a,*], Aleksey Lomakin [a], Sonoko Kanai [b], Rainer Alex [b], Sara Belli [c], Massimiliano Donzelli [c], George B. Benedek [a,d,e]

[a] Materials Processing Center, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
[b] Therapuetic Modality, Roche Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070 Basel, Switzerland
[c] Pharmaceutical Science, Roche Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070 Basel, Switzerland
[d] Department of Physics, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139, USA
[e] Center for Materials Science and Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 30 July 2016
Accepted 26 August 2016
Available online 27 August 2016

Keywords:
Lipidated peptide
Incretin
GLP1
Oligomerization
Albumin binding
Surface plasmon resonance

ABSTRACT

Hybrid incretin peptides are a new generation of drugs for the treatment of diabetes and obesity. Despite their biological potency, the effectiveness of these peptides as drugs is limited by their short circulation time in blood (typically within minutes). In this work, we show that lipid conjugated forms of a GLP-1/GIP/glucagon hybrid peptides stay in circulation for hours. We studied the oligomerization and albumin-binding of the unconjugated hybrid peptide as well as its lipidated variants. These lipidated peptides differ in the N-terminal mutation, the position of lipidation and the linkage to lipid. We found that these lipidated peptides form stable oligomers at concentrations above 1 mg/mL. This concentration range is relevant to formulation and storage of the peptides. We observed no binding between the peptide oligomers and human serum albumin. However, at the expected therapeutic concentration range (~10–100 ng/mL), the oligomers dissociate into monomers. The monomers of lipidated peptides bind to albumin. We have determined the dissociation constants of binding between the lipidated peptides and serum albumin. The dissociation constants of albumin-binding of our lipidated peptides are all very close and similar to that of the fatty acid binding of albumin. Our findings suggest that the monomeric lipidated peptides bind to HSA mainly by the fatty acid chain. Therefore, albumin binding is likely to be a universal mechanism of the prolonged circulating duration of lipidated pharmaceutical peptides.

1. Introduction

Peptides naturally occur in most organisms and play crucial biological roles, e.g., as hormones, toxin, and antibiotics [1]. Because of the versatile physiological functions of bioactive peptides, natural peptides and their synthetic analogues have become a major class of pharmaceutical molecules [2–4]. Bioactive peptides generally have high potency and selectivity, but their application as drugs is often limited by low metabolic stability, rapid clearance, low membrane permeability and sometimes low solubility [2–4]. One widely used approach to improve the pharmacokinetic properties of bioactive peptides is through lipidation of the peptides, i.e. covalent linking of the peptides to lipid conjugates [5–11]. The lipidated peptides often acquire superior pharmacological properties, e.g., higher metabolic stability, long circulating time, and the ability to interact with serum proteins and cell membranes. [7–14].

Although lipidation is widely used in the development of peptide drugs, the molecular basis for the long circulating lifetime of lipidated peptides is poorly understood. Two plausible mechanisms can explain the long duration of lipidated peptides in circulation [8,15]. First, the amphiphilic lipidated peptides may form micelle-like oligomers that are less susceptible to metabolic degradation and renal clearance. An alternative explanation is that the lipid moieties on the peptides may bind to serum proteins such as serum albumin. The peptide-albumin complex has long life-time in circulation due to reduced filtration by the kidney. Indeed, previous studies have shown that liraglutide, a lipidated GLP-1 agonist forms micelle-like oligomers [15] and also interacts with serum proteins [16]. Therefore, the predominant peptide species in the blood depend on the competition between oligomerization of the lipidated peptide and its interaction with serum albumin. Lipidated peptides differ in their amino acid sequence, the conjugated acyl group and the position of conjugation, and thereby could vary in their propensity to form oligomer as well as their binding affinity to serum albumin.

* Corresponding author at: Department of Chemistry and Biochemistry, 601 South College Road, Wilmington, NC 28403, USA.
E-mail address: wangyy@uncw.edu (Y. Wang).

http://dx.doi.org/10.1016/j.jconrel.2016.08.035
0168-3659/

Hence, different lipidated peptide may have different molecular basis for their long circulating lives. To understand the metabolism and pharmacokinetics of therapeutic lipidated peptides, it is necessary to determine in which form they exist in the circulation.

In this work, we studied the molecular mechanism of the prolonged circulating duration of several lipidated hybrid incretin peptides listed in Table S1. Natural incretins including glucagon-like peptide-1 (GLP-1) and the gastric inhibitory peptide (GIP) are gastrointestinal hormones. They lower the blood glucose level by increasing the glucose-dependent insulin secretion, inhibiting the glucagon secretion, and reducing gastric emptying [17–19]. These pharmacological effects make incretin peptides attractive drug candidates for the treatment of type-II diabetes and obesity [19–21]. In recent studies, a series of hybrid peptides with mixed sequences of both GLP-1 and GIP peptides have been developed [8,22,23]. The hybrid peptides exhibit co-agonism and enhanced glucose-dependent insulinotropic efficacy as well as additional fat-reducing efficacy. It had been demonstrated that such hybrid peptides have triple actions on the GLP-1, GIP and glucagon receptors [24]. These hybrid peptides may represent the inception of the development of the next-generation incretin drugs for the treatment of diabetes and obesity. However, the therapeutic effect of these incretin peptides are limited by their short circulating half-life in blood due to rapid degradation catalyzed by the dipeptidyl peptidase 4 (DPP-4) and fast renal clearance [21,25–27]. Lipidation has been employed to extend the duration of the therapeutic effects of the hybrid peptides [23].

Here, we show that the palmityolated GLP-1/GIP hybrid peptides have prolonged circulation time in rats. We have conducted an in-vitro study of oligomerization and albumin-binding of the unconjugated hybrid peptide (denoted hereafter as IP0, short for incretin peptide number 0) as well as its lipidated variant (denoted as IP1). The hybrid peptide is a triagonist with balanced potency for the glucagon, GIP, and GLP-1 receptors (GCGR and GLP-1R). The biological functions of this hybrid peptide and its lipidated counterparts have been previously demonstrated [8]. As shown in Table S1 in the supplementary material, IP0 consists of mixed amino acid sequence of GLP-1, GIP or glucagon and the C-terminal extension of exenatide. IP1 has the same amino acid sequence as IP0 with a palmityol group (C_{16}) linked to the ε-amino group of the C-terminal lysine through a glutamic acid residue. Previous studies have shown that mutations of the N-terminal histidine residue of GLP-1 analogues significantly affect their biological activity and pharmaceutical efficacy, and thereby are of great interest in the development of incretin drugs [28–32]. To examine the effects of these mutations on solution behavior of our lipidated incretin peptides, we have also used three mutants (H1Y, H1T and H1K) of IP1 in our study (respectively denoted as IP2, IP3 and IP4). These three substituted residues respectively have aromatic, polar and ionic side chains. In addition, we are interested in the effect of the position of lipidation. To this end, we have studied another IP1 variant (denoted as IP5) which has the conjugated palmitoyl group in the middle of the peptide (at the Y10K residue). Finally, we examined the role of the conjugation linkage by studying the IP1 variant (denoted IP6) with double glutamic acid residues between the peptide and the lipid as well as an additional C-terminal glycine residue. The primary structures of all these peptides are shown in Table S1.

The results of our studies show that all the lipidated hybrid incretin peptides can form oligomers at relatively high peptide concentrations (>~1 mg/mL) relevant to drug formulation. The peptide oligomers do not bind to human serum albumin (HSA). However, at a peptide concentration comparable to the pharmacologically active levels in human plasma (10–100 ng/mL), the peptides mainly exist in monomeric form and essentially all bind to HSA at physiological albumin concentrations. Therefore, our in-vitro studies suggest that palmityolated hybrid peptides would be in complex with serum albumin in vivo. Our studies also show that the N-terminal mutations, the position of lipid conjugation and the linker amino acid residues have only minor effect on the oligomerization and HSA-binding of the peptides. Since many of the drug candidates of the hybrid incretin peptides have similar amino acid sequence to our peptides, our study suggests that binding to serum proteins is a universal molecular basis for the long circulation of these peptides.

2. Materials and methods

2.1. Materials and solution preparation

The lyophilized hybrid peptides (IP0-6) were purchased from the custom peptide synthesis service provided by CS Bio Co., Menlo Park, CA. The purity of these peptides is above 96%, tested by reverse phase high performance liquid chromatography (HPLC) with a C8 column. The molecular weights of the monomeric peptides (IP0-6) measured by mass spectrometry are respectively 4338.90, 4706.19, 4732.29, 4671.51, 4697.31, 4543.59 and 4892.19 g/mol. The phosphate buffered saline (PBS, 1.7 mM KH_2PO_4, 10 mM Na_2HPO_4, 2.7 mM KCl and 137 mM NaCl, pH 7.4) were prepared using NaH_2PO_4 (Mallinckrodt Chemicals, St. Louis, MO), KH_2PO_4, KCl and NaCl (Sigma Aldrich, St. Louis, MO). All buffers were filtered through the membrane filter with the pore size 0.45 μm (Millipore, Bedford, MA). The peptide powder was dissolved in the PBS to prepare the stock solutions. The solution pH was adjusted to 9 with 1 M NaOH (Macron Fine Chemicals™) to facilitate dissolution of the peptides. Then, the pH was adjusted back to pH 7.4 using 1 M phosphoric acid (Mallinckrodt Chemicals). The peptide concentration was measured using a UV spectrophotometer (DU640, Beckman Coulter, Brea, CA). The extinction coefficients of the peptides (IP0-6) at 280 nm are respectively ε_{280} = 1.96, 1.80, 2.11, 1.82, 1.81, 1.54 and 1.75 L/g·cm, calculated from its amino acid sequence using the ProtParam tool on the ExPASy Bioinformatics Resource Portal (www.expasy.org) and adjusted by their total molecular weights. The peptide stock solutions were then diluted using PBS to desired concentrations. The isoelectric point of the lipidated peptides (IP1-6) are respectively pH 4.5, 3.6, 3.7, 4.4, 4.0 and 3.8, determined using capillary electrophoresis. The pH of solution was measured using a microelectrode (E5259, Sigma Aldrich, St. Louis, MO) with the accuracy of ±0.02 pH units.

Albumin from human serum (lyophilized powder, fatty acid free, globulin free, ≥99% by agarose gel electrophoresis) were purchased from Sigma Aldrich. Size-exclusion HPLC showed that the albumin contains about 8% of dimers and other oligomers. The albumin monomers were purified using a preparative chromatographic system (AKTA prime plus, Amersham Biosciences) and a size-exclusion column (packed with Sephacryl S-200, GE Healthcare). After purification, the monomer purity determined by HPLC is higher than 98%. The molecular weight, 66,472 g/mol, and the extinction coefficient, ε_{280} = 0.52 L/g·cm, of HSA were calculated from its amino acid sequence using the ProtParam tool on the ExPASy (www.expasy.org).

2.2. Pharmacokinetic (PK) studies

Three adult male Wistar rats weighting each approximately 230 g, received a single subcutaneous injection of 10 nmol/kg of the test peptide (IP5 and IP6 respectively). Plasma samples were obtained at 0.08, 0.25, 0.5, 1, 2, 4, 7, 24, 28, and 32 h after injection, and the peptide concentrations in plasma were determined by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Pharmacokinetic calculations (non-compartmental analysis) were performed by the Software Biobook (IDBS e-workbook Suite 8.2.0) using mean plasma concentrations data (n = 3 values per time-point). The following PK parameters were estimated: the maximum plasma concentration, C_{max}; the time taken to reach C_{max}, t_{max}; the circulating half-life, $t_{1/2}$; and the area under the concentration-time curve, AUC_{32h}, calculated from time = 0 h to the last observed time point (32 h).