US006514500B1

(12) **United States Patent**
**Bridon et al.**

(10) **Patent No.: US 6,514,500 B1**
(45) **Date of Patent: *Feb. 4, 2003**

(54) **LONG LASTING SYNTHETIC GLUCAGON LIKE PEPTIDE {GLP-!}**

(75) Inventors: **Dominique P. Bridon**, Outremont (CA); **Benoit L'Archeveque**, Laval (CA); **Alan M. Ezrin**, Moraga, CA (US); **Darren L. Holmes**, Montreal (CA); **Anouk Leblanc**, Montreal (CA); **Serge St. Pierre**, Ile Bizard (CA)

(73) Assignee: **Conjuchem, Inc.**, Montreal (CA)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/657,332**

(22) Filed: **Sep. 7, 2000**

**Related U.S. Application Data**

(60) Provisional application No. 60/159,783, filed on Oct. 15, 1999.

(51) **Int. Cl.**[7] ..... **A61K 39/385**; A61K 38/00; A01N 37/18

(52) **U.S. Cl.** ..... **424/193.1**; 424/195.11; 514/2

(58) **Field of Search** ..... 424/9.1, 488, 193.1; 514/2, 3, 6, 12, 18, 19; 530/303, 308, 399, 402, 409

(56) **References Cited**

U.S. PATENT DOCUMENTS

| | | | | |
|---|---|---|---|---|
| 5,118,666 | A | 6/1992 | Habener | 514/12 |
| 5,120,712 | A | 6/1992 | Habener | 514/12 |
| 5,493,007 | A | 2/1996 | Burnier et al. | 530/317 |
| 5,545,618 | A | 8/1996 | Buckley et al. | 514/12 |
| 5,574,008 | A | 11/1996 | Johnson et al. | 514/12 |
| 5,612,034 | A | 3/1997 | Pouletty et al. | 424/184.1 |
| 5,614,487 | A | 3/1997 | Battersby et al. | 514/2 |
| 5,614,492 | A | 3/1997 | Habener | 514/12 |
| 5,958,909 | A | 9/1999 | Habener | 514/120 |
| 5,981,488 | A | 11/1999 | Hoffmann | 514/12 |
| 6,133,235 | A | 10/2000 | Galloway et al. | 514/12 |
| 6,191,102 | B1 | 2/2001 | DiMarchi et al. | |

FOREIGN PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| EP | 0 602 290 | 6/1994 | A61K/47/48 |
| EP | 0 969 016 | 1/2000 | C07K/14/605 |
| WO | 9111457 | 8/1991 | C07K/7/34 |
| WO | 9325579 | 12/1993 | C07K/7/34 |
| WO | 9510302 | 4/1995 | A61K/39/395 |
| WO | 9808531 | 3/1998 | A61K/38/00 |
| WO | 9808871 | 3/1998 | C07K/14/605 |
| WO | 9808873 | 3/1998 | C07K/14/605 |
| WO | 9843658 | 10/1998 | A61K/38/00 |
| WO | 9924074 | 5/1999 | A61K/47/48 |
| WO | 9924075 | 5/1999 | A61K/47/48 |
| WO | 9929336 | 6/1999 | A61K/38/26 |
| WO | 9948536 | 9/1999 | A61K/47/48 |
| WO | 00/76550 | 12/2000 | A61K/47/48 |
| WO | 00/76551 | 12/2000 | A61K/47/48 |

OTHER PUBLICATIONS

Marburg et al., "Intorduction of the Maleimide Function onto Resin–Bound Peptides: A simple, High yield Process Useful for Discriminating among Several Lysines," Bioconjugate Chemistry, vol. 7, pp. 612–616 (1996).*
Regulatory Peptides, 1999, 79, 93–102.
U.S. patent application Ser. No. 09/623,618, Bridon et al., filed Sep. 2000.
Proc. Natl. Acad. Sci., 1983, 80, 5485–5489.
Nature, 1983, 302, 716–718.
Endocrinol., 1984, 115, 2176–2181.
Anti–Cancer Drugs, 1997, 8, 677–685.

* cited by examiner

*Primary Examiner*—James Housel
*Assistant Examiner*—Zachariah Lucas
(74) *Attorney, Agent, or Firm*—Morrison & Foerster LLP

(57) **ABSTRACT**

Modified insulinotropic peptides are disclosed. The modified insulinotropic peptides are capable of forming a peptidase stabilized insulinotropic peptide. The modified insulinotropic peptides are capable of forming covalent bonds with one or more blood components to form a conjugate. The conjugates may be formed in vivo or ex vivo. The modified peptides are administered to treat humans with diabetes and other related diseases.

**2 Claims, No Drawings**

# LONG LASTING SYNTHETIC GLUCAGON LIKE PEPTIDE {GLP-!}

This application claims the benefit under 35 U.S.C. §119(e) of United States provisional patent application No. 60/159,783 filed Oct. 15, 1999, which is hereby incorporated by reference in its entirety.

## FIELD OF THE INVENTION

This invention relates to modified insulinotropic peptides. In particular, this invention relates to modified glucagon like peptides and exendin peptides with long duration of action for the treatment of diabetes and other insulinotropic peptide related diseases, gastrointestinal function and activities associated with glucagon levels.

## BACKGROUND OF THE INVENTION

The insulinotropic peptide hormone glucagon-like peptide (GLP-1) has been implicated gas a possible therapeutic agent for the management of type 2 non-insulin-dependent diabetes mellitus as well as related metabolic disorders, such as obesity. Other useful insulinotropic peptides include exendin 3 and exendin 4. While useful, GLP-1, exendin 3 and exendin 4 suffer from limited duration of action associated with short plasma half-lifes in vivo, mainly due to rapid serum clearance and proteolytic degradation. The enzyme responsible for the degradation of GLP-1, dipeptidyl peptidase IV, has been identified. Extensive work has been done in attempts to inhibit the peptidase or to modify GLP-1 in such a way that its degradation is slowed down while still maintaining biological activity. Despite these extensive efforts, a long lasting, active GLP-1 has not been produced. As such, the diabetic community has a tremendous need for improved GLP-1, exendin 3 and exendin 4 peptides.

There is thus a need to modify GLP-1, exendin 3, exendin 4 and other insulinotropic peptides to provide longer duration of action in vivo, while maintaining their low toxicity and therapeutic advantages.

## SUMMARY OF THE INVENTION

In order to meet those needs, the present invention is directed to modified insulinotropic peptides (ITPs). This invention relates to novel chemically reactive derivatives of insulinotropic peptides that can react with available functionalities on cellular carriers including mobile blood proteins to form covalent linkages. Specifically, the invention relates to novel chemically reactive derivatives of insulinotropic peptides such as glucagon like peptide (GLP) and exendin 3 and exendin 4 that can react with available functionalities on mobile blood proteins to form covalent linkages. The invention also relates to novel chemically reactive derivatives or analogs of insulinotropic peptides that can react with available functionalities on mobile blood proteins to form covalent linkages.

The present invention relates to modified insulinotropic peptides comprising a reactive group which reacts with amino groups, hydroxyl groups or thiol groups on blood compounds to form stable covalent bonds.

The present invention relates to an insulinotropic hormone comprising a modified fragment of GLP-1 and derivatives thereof, especially GLP-1 (7-36) amide. The invention additionally pertains to the therapeutic uses of such compounds, and especially to the use of modified GLP-1 (7-36) amide for the treatment of maturity onset diabetes mellitus (type II diabetes).



The present invention further relates to modified Exendin 3 and Exendin 4 fragments and therapeutic uses of such compounds.

In particular, the present invention is directed to GLP-1 (1-36)-$Lys^{37}$ (ε-MPA)-$NH_2$; GLP-1 (1-36)-$Lys^{37}$ (ε-AAEA-AEEA-MPA)-$NH_2$; GLP-1 (7-36)-$Lys^{37}$ (ε-MPA)-$NH_2$; GLP-1 (7-36)-$Lys^{37}$-(ε-AEEA-AEEA-MPA)-$NH_2$; D-$Ala^8$ GLP1 (7-36)-$Lys^{37}$ (ε-MPA)-$NH_2$; Exendin-4 (1-39)-$Lys^{40}$ (ε-MPA)-$NH_2$; Exendin-4 (1-39)-$Lys^{40}$ (ε-AEEA-AEEA-MPA)-$NH_2$; Exendin-3 (1-39)-$Lys^{40}$ (ε-MPA)-$NH_2$; Exendin-3 (1-39)-$Lys^{40}$ (ε-AEEA-AEEA-MPA)-$NH_2$; $Lys^{26}$ (ε-MPA)GLP-1 (7-36)$NH_2$; GLP-1 (7-36)-EDA-MPA and Exendin-4 (1-39)-EDA-MPA.

The present invention further relates to compositions comprising the derivatives of the insulinotropic peptides and the use of the compositions for treating diabetes in humans.

The invention further pertains to a method for enhancing the expression of insulin which comprises providing to a mammalian pancreatic Beta-type islet cell an effective amount of the modified insulinotropic peptides disclosed above.

The invention further pertains to a method for treating maturity-onset diabetes mellitus which comprises administration of an effective amount of the insulinotropic peptides discussed above to a patient in need of such treatment.

The invention further pertains to the treatment of other insulinotropic peptide related diseases and conditions with the modified insulinotropic peptides of the invention.

## DETAILED DESCRIPTION OF THE INVENTION

Definitions

To ensure a complete understanding of the invention the following definitions are provided:

Insulinotropic Peptides: Insulinotropic peptides (ITPs) are peptides with insulinotropic activity. Insulinotropic peptides stimulate, or cause the stimulation of, the synthesis or expression of the hormone insulin. Such peptides include precursors, analogues, fragments of peptides such as Glucagon-like peptide, exendin 3 and exendin 4 and other peptides with insulinotropic activity.

Glucagon-Like Peptide: Glucagon-Like Peptide (GLP) and GLP derivatives are intestinal hormones which generally simulate insulin secretion during hyperglycemia, suppresses glucagon secretion, stimulates (pro) insulin biosynthesis and decelerates gastric emptying and acid secretion. Some GLPs and GLP derivatives promote glucose uptake by cells but do not simulate insulin expression as disclosed in U.S. Pat. No. 5,574,008 which is hereby incorporated by reference.

Exendin 3 and Exendin 4 Peptides: Exendin 3 and exendin 4 peptides and peptide derivatives are 39 amino acid peptides which are approximately 53% homologons to GLP-1 and have insulinotropic activity.

Reactive Groups: Reactive groups are chemical groups capable of forming a covalent bond. Such reactive agents are coupled or bonded to an insulinotropic peptide of interest to form a modified insulinotropic peptide. Reactive groups will generally be stable in an aqueous environment and will usually be carboxy, phosphoryl, or convenient acyl group, either as an ester or a mixed anhydride, or an imidate, thereby capable of forming a covalent bond with functionalities such as an amino group, a hydroxy or a thiol at the target site on mobile blood components. For the most part, the esters will involve phenolic compounds, or be thiol esters, alkyl esters, phosphate esters, or the like. Reactive groups include succinimidyl and maleimido groups.

Functionalities: Functionalities are groups on blood components to which reactive groups on modified insulinotropic peptides react to form covalent bonds. Functionalities include hydroxyl groups for bonding to ester reactive entities; thiol groups for bonding to malemides and maleimido groups, imidates and thioester groups; amino groups for bonding to carboxy, phosphoryl or acyl groups on reactive entities and carboxyl groups for bonding to amino groups. Such blood components include blood proteins.

Linking Groups: Linking groups are chemical moieties that link or connect reactive groups to ITPs. Linking groups may comprise one or more alkyl groups such as methyl, ethyl, propyl, butyl, etc. groups, alkoxy groups, alkenyl groups, alkynyl groups or amino group substituted by alkyl groups, cycloalkyl groups, polycyclic groups, aryl groups, polyaryl groups, substituted aryl groups, heterocyclic groups, and substituted heterocyclic groups. Linking groups may also comprise poly ethoxy aminoacids such as AEA ((2-amino)ethoxy acetic acid) or a preferred linking group AEEA ([2-(2-amino)ethoxy)]ethoxy acetic acid).

Blood Components: Blood components may be either fixed or mobile. Fixed blood components are non-mobile blood components and include tissues, membrane receptors, interstitial proteins, fibrin proteins, collagens, platelets, endothelial cells, epithelial cells and their associated membrane and membraneous receptors, somatic body cells, skeletal and smooth muscle cells, neuronal components, osteocytes and osteoclasts and all body tissues especially those associated with the circulatory and lymphatic systems. Mobile blood components are blood components that do not have a fixed situs for any extended period of time, generally not exceeding 5, more usually one minute. These blood components are not membrane-associated and are present in the blood for extended periods of time and are present in a minimum concentration of at least 0.1 $\mu$g/ml. Mobile blood components include serum albumin, transferrin, ferritin and immunoglobulins such as IgM and IgG. The half-life of mobile blood components is at least about 12 hours.

Protective Groups: Protective groups are chemical moieties utilized to protect peptide derivatives from reacting with themselves. Various protective groups are disclosed herein and in U.S. Pat. No. 5,493,007 which is hereby incorporated by reference. Such protective groups include acetyl, fluorenylmethyloxycarbonyl (FMOC), t-butyloxycarbonyl (BOC), benzyloxycarbonyl (CBZ), and the like. The specific protected amino acids are depicted in Table 1.

TABLE 1

NATURAL AMINO ACIDS AND THEIR ABBREVIATIONS

| Name | 3-Letter Abbreviation | 1-Letter Abbreviation | Protected Amino Acids |
|---|---|---|---|
| Alanine | Ala | A | Fmoc-Ala-OH |
| Arginine | Arg | R | Fmoc-Arg(Pbf)-OH |
| Asparagine | Asn | N | Fmoc-Asn(Trt)-OH |
| Aspartic acid | Asp | D | Asp(tBu)-OH |
| Cysteine | Cys | C | Fmoc-Cys(Trt) |
| Glutamic acid | Glu | E | Fmoc-Glu(tBu)-OH |
| Glutamine | Gln | Q | Fmoc-Gln(Trt)-OH |
| Glycine | Gly | G | Fmoc-Gly-OH |
| Histidine | His | H | Fmoc-His(Trt)-OH |
| Isoleucine | Ile | I | Fmoc-Ile-OH |
| Leucine | Leu | L | Fmoc-Leu-OH |
| Lysine | Lys | K | Fmoc-Lys(Mtt)-OH |
| Methionine | Met | M | Fmoc-Met-OH |
| Phenylalanine | Phe | F | Fmoc-Phe-OH |
| Proline | Pro | P | Fmoc-Pro-OH |

TABLE 1-continued

NATURAL AMINO ACIDS AND THEIR ABBREVIATIONS

| Name | 3-Letter Abbreviation | 1-Letter Abbreviation | Protected Amino Acids |
|---|---|---|---|
| Serine | Ser | S | Fmoc-Ser(tBu)-OH |
| Threonine | Thr | T | Fmoc-Thr(tBu)-OH |
| Tryptophan | Trp | W | Fmoc-Trp(Boc)-OH |
| Tyrosine | Tyr | Y | Boc-Tyr(tBu)-OH |
| Valine | Val | V | Fmoc-Val-OH |

Sensitive Functional Groups—A sensitive functional group is a group of atoms that represents a potential reaction site on an ITP peptide. If present, a sensitive functional group may be chosen as the attachment point for the linker-reactive group modification. Sensitive functional groups include but are not limited to carboxyl, amino, thiol, and hydroxyl groups.

Modified Peptides—A modified ITP is a peptide that has been modified by attaching a reactive group, and is capable of forming a peptidase stabilized peptide through conjugation to blood components. The reactive group may be attached to the therapeutic peptide either via a linking group, or optionally without using a linking group. It is also contemplated that one or more additional amino acids may be added to the therapeutic peptide to facilitage the attachment of the reactive group. Modified peptides may be administered in vivo such that conjugation with blood components occurs in vivo, or they may be first conjugated to blood components in vitro and the resulting peptidase stabalized peptide (as defined below) administered in vivo. The terms "modified therapeutic peptide" and "modified peptide" may be used interchangeably in this application.

Peptidase Stabilized ITP—A peptidase stabilized ITP is a modified peptide that has been conjugated to a blood component via a covalent bond formed between the reactive group of the modified peptide and the functionalities of the blood component, with or without a linking group. Peptidase stabilized peptides are more stable in the presence of peptidases in vivo than a non-stabilized peptide. A peptidase stabilized therapeutic peptide generally has an increased half life of at least 10–50% as compared to a non-stabalized peptide of identical sequence. Peptidase stability is determined by comparing the half life of the unmodified ITP in serum or blood to the half life of a modified counterpart therapeutic peptide in serum or blood. Half life is determined by sampling the serum or blood after administration of the modified and non-modified peptides and determining the activity of the peptide. In addition to determining the activity, the length of the ITP may also be measured by HPLC and Mass Spectrometry.

## DETAILED DESCRIPTION OF THE INVENTION

Taking into account these definitions the focus of this invention is to modify insulinotropic peptides to improve bio-availability, extend half-life and distribution through selective conjugation onto a protein carrier but without modifying their remarkable therapeutic properties. The carrier of choice (but not limited to) for this invention would be albumin conjugated through its free thiol by a insulinotropic peptide derivatized with a maleimide moiety.

1. Insulinotropic Peptides

A. GLP-1 and Its Derivatives The hormone glucagon is known to be synthesized as a high molecular weight precursor molecule which is subsequently proteolytically

cleaved into three peptides: glucagon, glucagon-like peptide 1 (GLP-1), and glucagon-like peptide 2 (GLP-2). GLP-1 has 37 amino acids in its unprocessed form as shown in SEQ ID NO: 1. Unprocessed GLP-1 is essentially unable to mediate the induction of insulin biosynthesis. The unprocessed GLP-1 peptide is, however, naturally converted to a 31-amino acid long peptide (7-37 peptide) having amino acids 7-37 of GLP-1 ("GLP-1 (7-37)") SEQ ID NO:2. GLP-1 (7-37) can also undergo additional processing by proteolytic removal of the C-terminal glycine to produce GLP-1 (7-36) which also exists predominantly with the C-terminal residue, arginine, in amidated form as argininеamide, GLP-1 (7-36) amide. This processing occurs in the intestine and to a much lesser extent in the pancreas, and results in a polypeptide with the insulinotropic activity of GLP-1 (7-37).

A compound is said to have an "insulinotropic activity" if it is able to stimulate, or cause the stimulation of, the synthesis or expression of the hormone insulin. The hormonal activity of GLP-1 (7-37) and GLP-1 (7-36) appear to be specific for the pancreatic beta cells where it appears to induce the biosynthesis of insulin. The glucagon-like-peptide hormone of the invention is useful in the study of the pathogenesis of maturity onset diabetes mellitus, a condition characterized by hyperglycemia in which the dynamics of insulin secretion are abnormal. Moreover, the glucagon-like peptide is useful in the therapy and treatment of this disease, and in the therapy and treatment of hyperglycemia.

Peptide moieties (fragments) chosen from the determined amino acid sequence of human GLP-1 constitute the starting point in the development comprising the present invention. The interchangeable terms "peptide fragment" and "peptide moiety" are meant to include both synthetic and naturally occurring amino acid sequences derivable from a naturally occurring amino acid sequence.

The amino acid sequence for GLP-1 has been reported by several researchers (Lopez, L. C., et al., Proc. Natl. Acad. Sci., USA 80:5485–5489 (1983); Bell, G. I., et al., Nature 302:716–718 (1983); Heinrich, G., et al., Endocrinol. 115:2176–2181 (1984)). The structure of the preproglucagon mRNA and its corresponding amino acid sequence is well known. The proteolytic processing of the precursor gene product, proglucagon, into glucagon and the two insulinotropic peptides has been characterized. As used herein, the notation of GLP-1 (1-37) refers to a GLP-1 polypeptide having all amino acids from 1 (N-terminus) through 37 (C-terminus). Similarly, GLP-1 (7-37) refers to a GLP-1 polypeptide having all amino acids from 7 (N-terminus) through 37 (C-terminus). Similarly, GLP-1 (7-36) refers to a GLP-1 polypeptide having all amino acids from number 7 (N-terminus) through number 36 (C-terminus).

In one embodiment, GLP-1 (7-36) and its peptide fragments are synthesized by conventional means as detailed below, such as by the well-known solid-phase peptide synthesis described by Merrifield, J. M. (Chem. Soc. 85:2149 (1962)), and Stewart and Young (Solid Phase Peptide Synthesis (Freeman, San Francisco, 1969), pages 27–66), which are incorporated by reference herein. However, it is also possible to obtain fragments of the proglucagon polypeptide, or of GLP-1, by fragmenting the naturally occurring amino acid sequence, using, for example, a proteolytic enzyme. Further, it is possible to obtain the desired fragments of the proglucagon peptide or of GLP-1 through the use of recombinant DNA technology, as disclosed by Maniatis, T., et al., Molecular Biology: A Laboratory Manual, Cold Spring Harbor, N.Y. (1982), which is hereby incorporated by reference.

The present invention includes peptides which are derivable from GLP-1 such as GLP-1 (1-37) and GLP-1 (7-36). A peptide is said to be "derivable from a naturally occurring amino acid sequence" if it can be obtained by fragmenting a naturally occurring sequence, or if it can be synthesized based upon a knowledge of the sequence of the naturally occurring amino acid sequence or of the genetic material (DNA or RNA) which encodes this sequence.

Included within the scope of the present invention are those molecules which are said to be "derivatives" of GLP-1 such as GLP-1 (1-37) and especially GLP-1 (7-36). Such a "derivative" has the following characteristics: (1) it shares substantial homology with GLP-1 or a similarly sized fragment of GLP-1; (2) it is capable of functioning as an insulinotropic hormone and (3) using at least one of the assays provided herein, the derivative has either (i) an insulinotropic activity which exceeds the insulinotropic activity of either GLP-1, or, more preferably, (ii) an insulinotropic activity which can be detected even when the derivative is present at a concentration of $10^{-10}$ M, or, most preferably, (iii) an insulinotropic activity which can be detected even when the derivative is present at a concentration of $10^{-11}$ M.

A derivative of GLP-1 is said to share "substantial homology" with GLP-1 if the amino acid sequences of the derivative is at least 80%, and more preferably at least 90%, and most preferably at least 95%, the same as that of GLP-1 (1-37).

The derivatives of the present invention include GLP-1 fragments which, in addition to containing a sequence that is substantially homologous to that of a naturally occurring GLP-1 peptide may contain one or more additional amino acids at their amino and/or their carboxy termini. Thus, the invention pertains to polypeptide fragments of GLP-1 that may contain one or more amino acids that may not be present in a naturally occurring GLP-1 sequence provided that such polypeptides have an insulinotropic activity which exceeds that of GLP-1. The additional amino acids may be D-amino acids or L-amino acids or combinations thereof.

The invention also includes GLP-1 fragments which, although containing a sequence that is substantially homologous to that of a naturally occurring GLP-1 peptide may lack one or more additional amino acids at their amino and/or their carboxy termini that are naturally found on a GLP-1 peptide. Thus, the invention pertains to polypeptide fragments of GLP-1 that may lack one or more amino acids that are normally present in a naturally occurring GLP-1 sequence provided that such polypeptides have an insulinotropic activity which exceeds that of GLP-1.

The invention also encompasses the obvious or trivial variants of the above-described fragments which have inconsequential amino acid substitutions (and thus have amino acid sequences which differ from that of the natural sequence) provided that such variants have an insulinotropic activity which is substantially identical to that of the above-described GLP-1 derivatives. Examples of obvious or trivial substitutions include the substitution of one basic residue for another (i.e. Arg for Lys), the substitution of one hydrophobic residue for another (i.e. Leu for Ile), or the substitution of one aromatic residue for another (i.e. Phe for Tyr), etc.

In addition to those GLP-1 derivatives with insulinotropic activity, GLP-1 derivatives which stimulate glucose uptate by cells but do not stimulate insulin expression or secretion are within the scope of this invention. Such GLP-1 derivatives are described in U.S. Pat. No. 5,574,008.

GLP-1 derivatives which stimulate glucose uptake by cells but do not stimulate insulin expression or secretion which find use in the invention include:

$R_1$-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Xaa-Gly-Arg-$R_2$ (SEQ ID NO:3) wherein $R_1$ is selected from a) $H_2N$; b) $H_2N$-Ser; c) $H_2N$-Val-Ser; d) $H_2N$-Asp-Val-Ser; e) $H_2N$-Ser-Asp-Val-Ser (SEQ ID NO:4); f) $H_2N$-Thr-Ser-Asp-Val-Ser (SEQ ID NO:5); g) $H_2N$-Phe-Thr-Ser-Asp-Val-Ser (SEQ ID NO:6); h) $H_2N$-Thr-Phe-Thr-Ser-Asp-Val-Ser (SEQ ID NO:7); i) $H_2N$-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser (SEQ ID NO:8); j) $H_2N$-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser (SEQ ID NO:9); or, k) $H_2N$-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser (SEQ ID NO:10). In the peptide, X is selected from Lys or Arg and $R_2$ is selected from $NH_2$, OH, Gly-$NH_2$, or Gly-OH. These peptides are C-terminal GLP-1 fragments which do not have insulinotropic activity but which are nonetheless useful for treating diabetes and hyperglycemic conditions as described in U.S. Pat. No. 5,574,008.

B. Exendin 3 and Exendin 4 Peptides

Exendin 3 and Exendin 4 are 39 amino acid peptides (differing at residues 2 and 3) which are approximately 53% homologous to GLP-1 and find use as insulinotropic agents.

The Exendin-3 [SEQ ID No:11] sequence is HSDGTFTSDLSKQMEEEAVRLFIEWLKNGG PSSGAPPPS and

The Exendin-4 [SEQ ID No:12] sequence is HGEGTFTSDLSKQMEEEAVRLFIEWLKNGG PSSGAPPPS.

The invention also encompasses the insulinotropic fragments of exendin-4 comprising the amino acid sequences: Exendin-4 (1-31) [SEQ ID No:13] HGEGTFTSDLSKQMEEAVR LFIEWLKNGGPY and Exendin-4 (1-31) [SEQ ID No:14] HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGY.

The invention also encompasses the inhibitory fragment of exendin-4 comprising the amino acid sequence:

Exendin-4(9-39) [SEQ ID No:15] DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS

Other insulinotropic peptides as presented in the Examples are shown as SEQ ID NO:16–22.

The present invention includes peptides which are derivable from the naturally occurring exendin 3 and exendin 4 peptides. A peptide is said to be "derivable from a naturally occurring amino acid sequence" if it can be obtained by fragmenting a naturally occurring sequence, or if it can be synthesized based upon a knowledge of the sequence of the naturally occurring amino acid sequence or of the genetic material (DNA or RNA) which encodes this sequence.

Included within the scope of the present invention are those molecules which are said to be "derivatives" of exendin 3 and exendin 4. Such a "derivative" has the following characteristics: (1) it shares substantial homology with exendin 3 or exendin 4 or a similarly sized fragment of exendin 3 or exendin 4; (2) it is capable of functioning as an insulinotropic hormone and (3) using at least one of the assays provided herein, the derivative has either (i) an insulinotropic activity which exceeds the insulinotropic activity of either exendin 3 or exendin 4, or, more preferably, (ii) an insulinotropic activity which can be detected even when the derivative is present at a concentration of $10^{-10}$ M, or, most preferably, (iii) an insulinotropic activity which can be detected even when the derivative is present at a concentration of $10^{-11}$ M.

A derivative of exendin 3 and exendin 4 is said to share "substantial homology" with exendin 3 and exendin 4 if the amino acid sequences of the derivative is at least 80%, and more preferably at least 90%, and most preferably at least 95%, the same as that of either exendin 3 or 4 or a fragment of exendin 3 or 4 having the same number of amino acid residues as the derivative.

The derivatives of the present invention include exendin 3 or exendin 4 fragments which, in addition to containing a sequence that is substantially homologous to that of a naturally occurring exendin 3 or exendin 4 peptide may contain one or more additional amino acids at their amino and/or their carboxy termini. Thus, the invention pertains to polypeptide fragments of exendin 3 or exendin 4 that may contain one or more amino acids that may not be present in a naturally occurring exendin 3 or exendin 4 sequences provided that such polypeptides have an insulinotropic activity which exceeds that of exendin 3 or exendin 4.

Similarly, the invention includes exendin 3 or exendin 4 fragments which, although containing a sequence that is substantially homologous to that of a naturally occurring exendin 3 or exendin 4 peptide may lack one or more additional amino acids at their amino and/or their carboxy termini that are naturally found on a exendin 3 or exendin 4 peptide. Thus, the invention pertains to polypeptide fragments of exendin 3 or exendin 4 that may lack one or more amino acids that are normally present in a naturally occurring exendin 3 or exendin 4 sequence provided that such polypeptides have an insulinotropic activity which exceeds that of exendin 3 or exendin 4.

The invention also encompasses the obvious or trivial variants of the above-described fragments which have inconsequential amino acid substitutions (and thus have amino acid sequences which differ from that of the natural sequence) provided that such variants have an insulinotropic activity which is substantially identical to that of the above-described exendin 3 or exendin 4 derivatives. Examples of obvious or trivial substitutions include the substitution of one basic residue for another (i.e. Arg for Lys), the substitution of one hydrophobic residue for another (i.e. Leu for Ile), or the substitution of one aromatic residue for another (i.e. Phe for Tyr), etc.

2. Modified Insulinotropic Peptides

This invention relates to modified insulinotropic peptides and their derivatives. The modified insulinotropic peptides of the invention include reactive groups which can react with available reactive functionalities on blood components to form covalent bonds. The invention also relates to such modifications, such combinations with blood components and methods for their use. These methods include extending the effective therapeutic in vivo half life of the modified insulinotropic peptides.

To form covalent bonds with the functional group on a protein, one may use as a chemically reactive group (reactive entity) a wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable at the levels required to modify the insulinotropic peptides. While a number of different hydroxyl groups may be employed in these linking agents, the most convenient would be N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS) and maleimidopropionic acid (MPA).

Primary amines are the principal targets for NHS esters as diagramed in the schematic below." Accessible α-amine groups present on the N-termini of proteins react with NHS esters. However, α-amino groups on a protein may not be desirable or available for the NHS coupling. While five amino acids have nitrogen in their side chains, only the ϵ-amine of lysine reacts significantly with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide as demonstrated in the schematic

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.