

FALL 2015 • Volume 14/Issue 3 • ISSN 1538-8786

# BioProcessing

## JOURNAL

*Trends & Developments in BioProcess Technology*

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# Glucagon-Like Peptide 1 Synthesis for Use in Human Diabetes Treatment

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## Introduction

**T**ype 2 diabetes is a major risk factor for cardiovascular disease-related morbidity and mortality. There are several therapies for type 2 diabetes management, but optimal glycemic control has not been achieved yet. A large number of patients fail to attain an ideal glycemic target, and only a few drugs have demonstrated effective control of glycated hemoglobin (HbA<sub>1c</sub>) numbers below 7%. The biggest hurdles for implementing long-term, effective therapies are hypoglycemia and weight gain. Most pharmaceuticals currently available act to increase insulin availability through administration, secretion, or by increasing insulin sensitivity. Others act by delaying the delivery and absorption of carbohydrates from the gastrointestinal (GI) tract or by increasing urinary glucose excretion. Recent advances in type 2 diabetes management include the clinical development of dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists.<sup>[1]</sup>

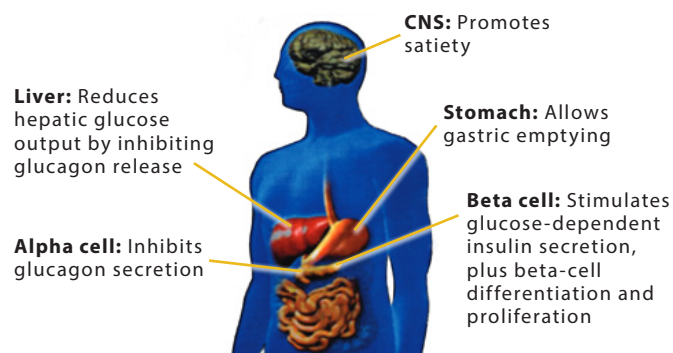
GLP-1 belongs to the hormonal family of incretins that enhance the secretion of insulin. Incretins lower blood glucose levels by stimulating pancreatic beta cells to release increased amounts of insulin. The two primary incretin hormones are GLP-1 and glucose-dependent insulintropic polypeptide (GIP), also known as gastric inhibitory polypeptide. Both GLP-1 and GIP are rapidly cleaved by DPP-4. GLP-1 is a product of a precursor molecule called pre-proglucagon, a polypeptide which is split to produce many hormones including glucagon. As they have the same origin, these hormones share some similarities, and hence the name "glucagon-like."

## About GLP-1

In the lining of the small intestines are L-cells, which are a major producer of GLP-1. The pancreas and the central nervous system (CNS) also secrete this hormone in smaller quantities. GLP-1 stimulates the release of insulin from the pancreas. It also increases the volume of beta cells in the pancreas that produce insulin, and regulates the release of glucagon. GLP-1 acts on appetite centers in the brain, slowing the stomach's emptying process and increasing the feeling of fullness during and between meals.

Differential processing of proglucagon in the intestinal epithelial endocrine L-cells produces the 30-amino acid peptide hormone GLP-1. The metabolism of GLP-1 in the body is extremely rapid and the peptide gets inactivated by DPP-4, even before leaving the gut.<sup>[2]</sup> Glucagon is a key hormone in glucose metabolism and homeostasis. It regulates blood glucose by decreasing glycolysis and increasing the rate of gluconeogenesis. It counter-regulates the hormone of insulin by raising plasma glucose levels in response to insulin-induced hypoglycemia. Glucagon is known to play an important role in initiating and maintaining hyperglycemic conditions in diabetes and suppressing the plasma glucagon level.<sup>[3]</sup>

GLP-1 is a potent stimulator of glucose-dependent insulin release and plays an important role in the gastric emptying (or motility process) and the suppression of plasma glucagon levels. GLP-1 is speculated to be involved in the stimulation of glucose disposal in peripheral tissues, independent of insulin actions, and is involved in the growth promotion of intestinal epithelium.<sup>[4]</sup> It might even regulate pituitary and hypothalamus axis, affecting the secretion of luteinising hormones, thyroid-stimulating hormones, oxytocin, corticotrophin-releasing hormones, and vasopressin. GLP-1 can also affect the proliferation and apoptosis of beta cells in the pancreas (**Figure 1**).



**FIGURE 1. Multiple sites of action for GLP-1.**

Image courtesy of the American Diabetes Association and John Buse, MD.<sup>[5]</sup>

**TABLE 1.** GLP-1 receptor agonist pharmaceuticals.\*

Drug Name	Developer/Manufacturer	Dosage	Plasma Half-Life	Status
Albiglutide (Esperzan®, Tanzeum®)	GlaxoSmithKline	Injectable (30-50 mg)	~5 days	Licensed in USA and EU
Dulaglutide (Trulicity™)	Eli Lilly	Injectable (0.75–1.5 mg)	~4.7 days	Licensed in USA
Exenatide (Byetta®)	AstraZeneca	Subcutaneous (5–10 mcg)	2.4 hours	Licensed in USA
Liraglutide (Victoza®)	Novo Nordisk	Subcutaneous (1.2–1.8 mg)	11–15 hours	Licensed in USA
Lixisenatide (Lyxumia®)	Sanofi-Aventis	Injectable (20 mcg)	2.7–4.3 hours	Licensed in EU, Mexico, Australia, Japan
Semaglutide	Novo Nordisk	Being determined	~160 hours	Late stage clinical trials

\*Table courtesy of the American Chemical Society and adapted with permission from Mandahar and Ahn.<sup>[7]</sup>

GLP-1 is co-secreted with GLP-2. They stimulate intestinal growth and upregulate villus height in the GI tract. The GLP-2's primary site of action starts in the stomach and ends in the colon. It has an essential function in nutrient homeostasis<sup>[6]</sup> by increasing stimuli in the GI tract, which leads to incremental nutrient assimilation. The pharmaceutical industry has developed drugs that mimic the GLP-1 targeting control of glucose levels in type 2 diabetes. Weight loss helps increase GLP-1 levels leading to improved glucose control in type 2 diabetes. Lower levels of GLP-1 in the body lead to obesity, feelings of hunger, and an empty stomach, which makes the individual eat more.

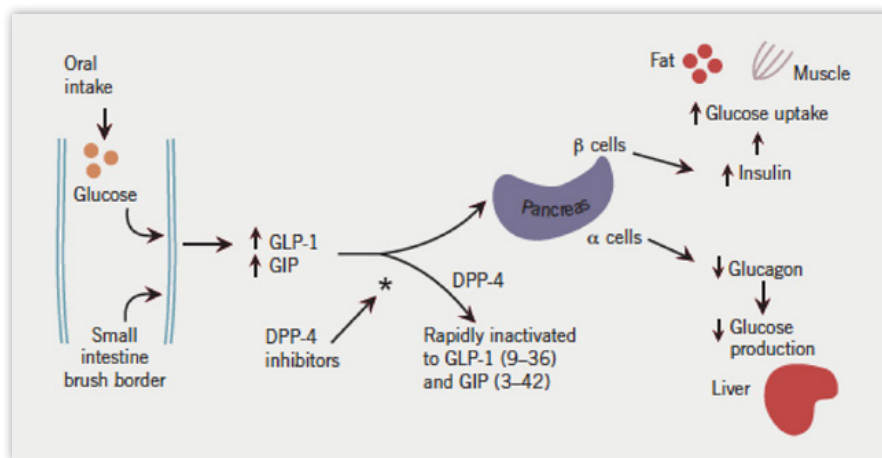
### The GLP-1 Class of Pharmaceuticals

Incretin-based GLP-1 receptor agonist therapies are a new class of injectable drugs and a novel option in the treatment of type 2 diabetes. They mimic the action of GLP-1 and increase the incretin effect in patients to stimulate the release of insulin. Incretin-based GLP-1 therapies have additional actions such as reducing glucagon, slowing down gastric emptying, and inducing the sense of satiety. In clinical practice, these therapies are associated

with significant reductions in HbA<sub>1c</sub>, weight loss, and a low hypoglycemia risk. Three of the incretin GLP-1 receptor agonist-based therapies are described in this paper. These and other GLP-1 receptor agonists are shown in **Table 1**.

**Exenatide** (exendin-4) is a reptilian hormone isolated from the saliva of the Gila monster (*Heloderma suspectum*) which acts as a GLP-1 mimic. The 39 amino acid-containing peptide has been approved as a monotherapy for the treatment of type 2 diabetes. Exenatide is marketed under the trade name Byetta by AstraZeneca. It enhances insulin production and has 53% sequence identity to GLP-1. Because of its shorter 2.4-hour half-life, exenatide requires a twice-a-day dosing regimen and must be taken with metformin (and for some patients, sulfonylurea too).

Clinical trials showed that exenatide, when used with other anti-diabetic drugs, reduced A1C by 0.4–1.5% in diabetic patients whose HbA<sub>1c</sub> was not controlled by metformin or sulfonylurea drugs alone. Weight loss was also seen along with good glycemic control. Exenatide was well-tolerated in patients with mild to moderate GI side-effects. Rates of hypoglycemia were relatively low in these studies (as illustrated in **Figure 2**).<sup>[5]</sup>



**FIGURE 2.** Oral glucose stimulates the release of the endogenous incretins GLP-1 and GIP. These stimulate insulin release while inhibiting the release of glucagon, resulting in lower blood glucose. They are rapidly inactivated by DPP-4, inhibitors that prolong the action of endogenous incretins, and enhance the first-phase insulin response.

Image courtesy of Miles Fisher, MD.

**Liraglutide** is a GLP-1 analog having a 97% sequence similarity to GLP-1. It is marketed under the trade name Victoza by Novo Nordisk. The amino acid arginine is replaced by lysine at the 34<sup>th</sup> position, and the addition of a C16 fatty acid chain to lysine 26 via glutamyl spacer makes it more stable, enables it to be a once-a-day dose, and prolongs its action to over 24 hours. The fatty acid side chain facilitates heptamer formation, thus increasing the stability and the binding to the albumin. The safety and efficacy of liraglutide was studied in detail in phase 3 LEAD trials ("liraglutide effect and action in diabetes"). It was demonstrated that liraglutide effectively improved glycemic control in diabetic patients when used as a monotherapy or in combination with other oral anti-diabetic drugs.<sup>[8]</sup> It seemed to decrease patient body mass, and the drug was generally well-tolerated apart from some transient nausea noted by patients in early trials. Rates of hypoglycemia seemed to be low in these trials. However, frequency of hypoglycemia increased with the use of other anti-diabetic drugs (metformin and sulfonylurea). It was reported that in direct comparison to the twice-daily exenatide, the once-a-day liraglutide was significantly more effective in controlling the glycemic index. Weight reduction was comparable for both. Fasting plasma glucose was significantly reduced with liraglutide treatment.

**Exenatide LAR** (long-acting release) from Amylin/Eli Lilly, branded as Bydureon<sup>®</sup> (exenatide once-a-week dose), was more effective when compared to the twice-daily Byetta (exenatide) dose.<sup>[9]</sup>

## Discussion

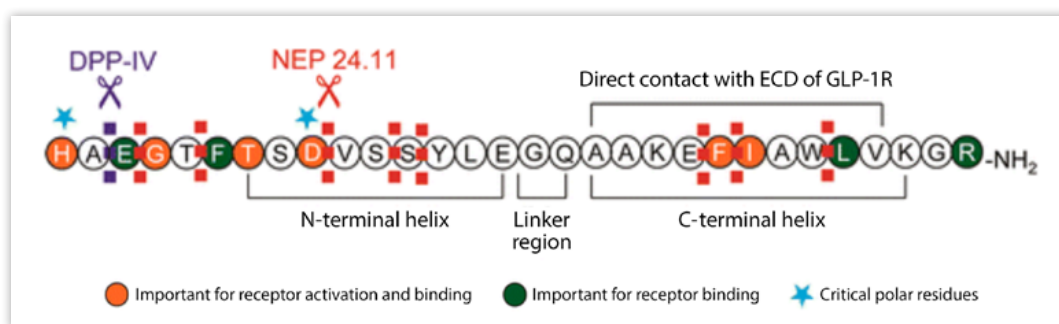
In hypoglycemia, GLP-1 reduces the brain glucose concentration. Further effects include an increase in the net blood brain clearance and the brain metabolism, but it is not known whether they depend on the prevailing plasma glucose (PG).<sup>[10]</sup>

The GLP-1 peptide is a post-translational product of glucagon, which is a precursor of many glucagon-related peptides. These are two equipotent forms of GLP-1, GLP-1 (7-36)-NH<sub>2</sub> and GLP-1 (7-37)—the first one being more abundant—which binds to and activates the GLP-1 receptor. The GLP-1 receptor (or GLP-1R) belongs to the

class B family of G-protein-coupled receptors (GPCRs) and carries out its regulatory functions. GLP-1R is found in many organs like the pancreas, kidney, GI tract, and brain, but is highly expressed in the pancreas. Activation of the receptor in beta cells of the pancreas (islet cells) induces an increase of the cAMP level and the Ca<sup>2+</sup> concentration, resulting in exocytosis of insulin in a glucose-dependent manner. GLP-1 inhibits glucagon secretion by 50% through modulation of calcium channel activity.<sup>[11, 12]</sup>


A conformational change is triggered when GLP-1 binds to the receptor leading to the activation of the related G-protein. This prompts the exchange of GDP that is bound to the  $\alpha$ -subunit of the G-protein to GTP, followed by the disassociation of the G-protein complex. The activated  $\alpha$ -subunit separates from the G-protein and interacts with effector systems, which finally results in an increase of secondary messengers like cAMP and Ca<sup>2+</sup>.<sup>[11, 13, 14]</sup> It appears (based on the experimental data in research papers) that His7, Gly10, Phe12, Thr13, Asp15, Phe28, and Ile29 play an important role in receptor binding, as a marked decrease in binding affinity was caused by each Ala substitution. Also, His7, Gly10, Asp15, and Phe28 seemed to be essential for receptor activation, as their Ala substitutions resulted in a substantial decrease in cAMP production. On the basis of these results, it is speculated that the N-terminal region of GLP-1 is more critical for activating the receptor<sup>[13, 14]</sup>, whereas the C-terminal region mainly contributes to receptor binding. Many residues in the N-terminal region are also important and considered as essential for receptor interaction. **Figure 3** shows the structure-activity relationship for GLP-1 agonists and the impact of the various amino acids on receptor binding.

His7 is found to be a crucial residue for both receptor binding and activation, as evidenced by the alanine scanning studies.<sup>[15]</sup> A negatively charged residue is necessary for receptor binding activity at position 15. Substitution of Asp15 by Arg was found to significantly reduce receptor binding and activity (710- and 90-fold, respectively). Val 16 could be replaced with a lipophilic amino acid without impacting receptor interaction and activity. Substitution of Gly22 by Aib retained the binding affinity and potency of the receptor. Attachment of



**FIGURE 3.** Structure activity relationship for the GLP-1 agonists.

Image courtesy of the American Chemical Society and used with permission from Mandahar and Ahn.<sup>[7]</sup>

A man with short brown hair, wearing black-rimmed glasses, a white dress shirt, a red tie, and a dark suit jacket. He is looking directly at the camera with a slight smile. The background is a blurred office setting with large windows.

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fatty acids to the epsilon amino group of Lys26 resulted in a prolonged half-life and increased stability<sup>[16]</sup> against degradation by DPP-4.

### Producing the Synthesized GLP-1

At American Peptide Company and Bachem, the general procedure employed to make this human GLP-1 was by Fmoc (9-fluorenylmethoxycarbonyl)-based solid-phase synthesis starting with Fmoc-glycine linked to an insoluble carrier resin. The peptide was assembled in a stepwise manner, coupling sequential amino acids from the C-terminal glycine to the N-terminus of the sequence. Each step consisted of Fmoc cleavage with piperidine, washing the resin, coupling the next Fmoc amino acid, and then removing unspent reagents. During the synthesis, standard reagents such as HBTU (O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium

hexafluorophosphate), and DIC/HOBt (diisopropyl carbodiimide in combination with 1-hydroxybenzotriazole) were used for coupling the Fmoc amino acids. After cleavage from the resin, the crude GLP-1 was purified using reverse-phase, high-pressure liquid chromatography (HPLC) to extract 98% pure material. Standard Fmoc-based solid-phase synthesis yields the trifluoroacetate of the peptide, which can be converted into other salt forms (acetate, hydrochloride, etc.) by ion exchange.

### In Summary

It would not be an exaggeration to say that peptide analogs are becoming dominant agents in the management of both type 1 and type 2 diabetes. GLP-1 agonist peptides against type 2 diabetes are promising targets for patients with poor HbA<sub>1c</sub> control. It is expected that the unique characteristics of GLP-1 peptides will continue to advance the treatment options available to diabetes patients.

### References


- [1] Author(s) not listed. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*, 1998; 352(9131): 837–53. [http://dx.doi.org/10.1016/S0140-6736\(98\)07019-6](http://dx.doi.org/10.1016/S0140-6736(98)07019-6). Erratum in: *Lancet*, 1999; 354(9178): 602. [http://dx.doi.org/10.1016/S0140-6736\(05\)77965-4](http://dx.doi.org/10.1016/S0140-6736(05)77965-4).
- [2] Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*, 2007; 87(4): 1409–39. <http://dx.doi.org/10.1152/physrev.00034.2006>. PMID:17928588.
- [3] Runge S, Wulff BS, Madsen K, Brauner-Osborne H, Knudsen LB. Different domains of the glucagon and glucagon-like peptide-1 receptors provide the critical determinants of ligand selectivity. *Br J Pharmacol*, 2003; 138(5): 787–94. <http://dx.doi.org/10.1038/sj.bjp.0705120>. PMID:12642379, PMCID:PMC1573731.
- [4] Kieffer TJ, Habener JF. The glucagon-like peptides. *Endocr Rev*, 1999; 20(6): 876–913. <http://dx.doi.org/10.1210/edrv.20.6.0385>. PMID:10605628.
- [5] Dungan K, Buse JB. Glucagon-like peptide 1-based therapies for type 2 diabetes: a focus on exenatide. *Clin Diabetes*, 2005; 23(2): 56–62. <http://dx.doi.org/10.2337/diaclin.23.2.56>.
- [6] Dong CX, Brubaker PL. Ghrelin, the proglucagon-derived peptides and peptide YY in nutrient homeostasis. *Nat Rev Gastroenterol Hepatol*, 2012; 9(12): 705–15. <http://dx.doi.org/10.1038/nrgastro.2012.185>. PMID:23026903.
- [7] Manandhar B, Ahn J-M. Glucagon-like peptide-1 (GLP-1) analogs: recent advances, new possibilities, and therapeutic implications. *J Med Chem*, 2015; 58(3): 1020–37. <http://dx.doi.org/10.1021/jm500810s>.
- [8] Dicker D. DPP-4 Inhibitors: impact on glycemic control and cardiovascular risk factors. *Diabetes Care*, 2011; 34(S2): 276–78. <http://dx.doi.org/10.2337/dc11-s229>.
- [9] Garber AJ. Long-acting glucagon-like peptide 1 receptor agonists: a review of their efficacy and tolerability. *Diabetes Care*, 2011; 34(S2): 279–84. <http://dx.doi.org/10.2337/dc11-s231>.
- [10] Gejl M, Lerche S, Egefjord L, Brock B, Moller N, Vang K, Rodell AB, Bibby BM, Holst JJ, Rungby J, Gjedde A. Glucagon-like peptide-1 (GLP-1) raises blood-brain glucose transfer capacity and hexokinase activity in human brain. *Front Neuroenergetics*, 2013; 5(2): 1–9. <http://dx.doi.org/10.3389/fnene.2013.00002>.
- [11] MacDonald PE, El-kholy W, Riedel MJ, Salapatek AMF, Light PE, Wheeler MB. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes*, 2002; 51(S3): 434–42. <http://dx.doi.org/10.2337/diabetes.51.2007.S434>. PMID:12475787.
- [12] Brubaker PL, Drucker DJ. Minireview: Glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. *Endocrinology*, 2004; 145(6): 2653–9. <http://dx.doi.org/10.1210/en.2004-0015>. PMID:15044356.
- [13] Diekmann M (Head of Global Marketing, Bachem Group). Pharmaceutical Chemistry PhD Dissertation, The University of Bonn, 2003, p 18.
- [14] De Marinis YZ, Salehi A, Ward CE, Zhang Q, Abdulkader F, Bengtsson M, Braha O, Braun M, Ramracheya R, Amisten S, Habib AM, Moritoh Y, Zhang E, Reimann F, Rosengren AH, Shibasaki T, Gribble F, Renstrom E, Seino S, Eliasson L, Rorsman P. GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca<sup>2+</sup> channel-dependent exocytosis. *Cell Metab*, 2010, 11(6): 543–53. <http://dx.doi.org/10.1016/j.cmet.2010.04.007>. PMID:20519125, PMCID:PMC4310935.
- [15] Donnelly D. The structure and function of the glucagon-like peptide-1 receptor and its ligands. *Br J Pharmacol*, 2012; 166(1): 27–41. <http://dx.doi.org/10.1111/j.1476-5381.2011.01687.x>.
- [16] Gupta V. Glucagon-like peptide-1 analogues: an overview. *Indian J Endocrinol Metab*, 2013; 17(3): 413–21. <http://dx.doi.org/10.4103/2230-8210.111625>. PMID:23869296, PMCID:PMC3712370.

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