ELSEVIER

Biochemical Pharmacology 64 (2002) 1753–1756

Biochemical Pharmacology

Short communication

A kinetic study of glucagon-like peptide-1 and glucagon-like peptide-2 truncation by dipeptidyl peptidase IV, *in vitro*

Anne-Marie Lambeir[a,*], Paul Proost[b], Simon Scharpé[a], Ingrid De Meester[a]

[a]*Laboratory of Medical Biochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1 S6, B-2610 Wilrijk, Belgium*

[b]*Laboratory of Molecular Immunology, Rega Institute for Medical Research, University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Received 20 June 2002; accepted 30 August 2002

Abstract

In vivo inactivation of glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) was found to be associated with the proteolytic removal of their N-terminal dipeptide by the ectopeptidase dipeptidyl peptidase IV (DPP IV). Previous studies suggested that the *in vivo* metabolism of GLP-1 is much faster than that of GLP-2. In this paper, we investigated the *in vitro* truncation of GLP-2 and GLP-1 by DPP IV. The slower conversion rate observed for GLP-2 compared to GLP-1 was due to an approximately 10-fold reduction in catalytic rate constant. The selectivity of DPP IV for the glucagon-like peptides was compared with data obtained for other natural substrates using the same enzyme source in identical conditions.

Keywords: Glucagon-like peptides; GLP-1; GLP-2; Incretins; Dipeptidyl peptidase IV; CD26

1. Introduction

Glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) are related neuropeptides produced by tissue-specific processing of the proglucagon precursor protein in the intestine [1]. Upon food intake, GLP-1 and GLP-2 are secreted by the endocrine L cells of the distal intestine. They promote nutrient assimilation and energy absorption in the gastrointestinal tract (reviewed in [2,3]). The first recognized physiological action of GLP-1 is to inhibit gastric emptying. It plays an important role in glucose homeostasis by its ability to lower blood glucose through activation of several independent mechanisms. GLP-1 acts as an incretin: it amplifies meal-induced insulin release in a glucose-dependent manner. It also increases insulin biosynthesis and suppresses the secretion of glucagon. GLP-1 participates in the processes leading to pancreatic islet proliferation and neogenesis. GLP-2 plays an essential role in gastric acid secretion, gastrointestinal motility, intestinal mucosa regeneration and permeability.

After their release, the activity of both peptides is controlled by the proteolytic removal of the N-terminal dipeptide (His-Ala) by dipeptidyl peptidase IV (DPP IV) [4–10]. The general result is activity loss, but there is evidence that the truncated peptides are not entirely without effect [11–13]. The *in vivo* half-life of intact GLP-1 in humans is 1–2 min (i.v.) [6]. The metabolism of GLP-2 is slower; in healthy volunteers, i.v. infused exogeneous GLP-2 is cleared with a half-life of 7 min [10]. Whereas a significant proportion of GLP-1 is already cleaved by DPP IV in the capillaries of the intestine [7], GLP-2 appears to be truncated less efficiently [10].

Both GLP-1 and GLP-2 have therapeutic potential for the treatment of diabetes and intestinal diseases, respectively. In defined experimental settings, inhibition of DPP IV improves the beneficial effect of either one of the glucagon-like peptides [14–18]. This may raise concerns

* Corresponding author. Tel.: +32-3-8202727; fax: +32-3-8202745.
E-mail address: anne-marie.lambeir@ua.ac.be (A.-M. Lambeir).

Abbreviations: DPP IV, dipeptidyl peptidase IV (EC 3.4.14.5); GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GRP, gastrin releasing peptide; I-TAC (CXCL11), interferon-inducible T cell α-chemoattractant; IP-10 (CXCL10), γ-interferon-inducible protein 10; MDC (CCL22), macrophage-derived chemokine; Mig (CXCL9), monokine induced by γ-interferon; NPY, neuropeptide Y; PACAP, pituitary adenylyl cyclase-activating peptide; RANTES (CCL5), regulated upon activation, normal T-lymphocyte expressed and presumably secreted; SDF1α (CXCL12), stromal cell-derived factor 1α; VIP, vasoactive intestinal peptide.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01415-6

about the use of DPP IV inhibitors, for example in the treatment of type 2 diabetes where intestinal proliferation due to increased GLP-2 levels is not desirable. In this context, it is important to thoroughly understand the interaction between the glucagon-like peptides and DPP IV.

Whereas the *in vitro* truncation by DPP IV is well documented for the incretins [4,5,19], little kinetic data have been reported for GLP-2 [9,10]. To eliminate uncertainties arising from differences in the estimation of the relative molecular mass and the specific activity of various DPP IV preparations, we decided to investigate the *in vitro* truncation of GLP-1 and GLP-2 side-by-side in well defined conditions. The physiological and medical importance of DPP IV substrates in general has been reviewed by Mentlein [20] and De Meester *et al.* [21,22]. We previously reported kinetic data on several peptides implicated in pancreatic insulin secretion and on a number of chemokines [23,24]. In this paper, we compare the selectivity of DPP IV for GLP-1 and GLP-2, mutually and in relation to these substrates.

2. Materials and methods

GLP-1, GLP-2, neurotensin and Phe-Pro were obtained from Bachem, BSA, Cyt *c*, Gly-Pro-*p*-nitroanilide and bradykinin from Sigma and GRP(3–27) was custom synthesized by Neosystem Labs.

DPP IV was purified from human seminal plasma as reported elsewhere [25]. The specific activity was 35 unit/mg, determined with Gly-Pro-*p*-nitroanilide (0.5 mM) in 40 mM Tris/HCl buffer, pH 8.3. The theoretical molecular mass of soluble DPP IV is 85,123 per subunit.

The truncation kinetics of GLP-1 and GLP-2 were measured at 37° in the reaction buffer containing 50 mM Tris/HCl buffer, pH 7.5 and 1 mM EDTA in the presence of 10 and 100 unit/L DPP IV, respectively. The concentration dependency of the truncation rate was determined between 5 and 120 μM of peptide (six concentrations, N = 3). The composition of the reaction mixture was determined with an Esquire LC Ion Trap mass spectrometer (Bruker) as described [23,24].

The molecular mass of the peptides was determined by gel filtration chromatography on a Superdex peptide HR10/30 column using the Äkta explorer (Amersham Biosciences). The dimensions of the column were 10 mm × 300 mm, the elution rate was 0.5 mL/min. Protein elution was monitored by measuring the absorbance at 215 and 280 nm. The peptide concentration in the sample was 100 μM in a volume of 100 μL. The buffers were 50 mM Tris/HCl buffer, pH 7.5, 1 mM EDTA for the native peptides and 30% acetonitrile, 0.1% TFA for the denatured peptides. The column was calibrated with the following polypeptides: BSA (64,000), Cyt *c* (12,500), GRP(3–27) (2660), neurotensin (1672), bradykinin (1060) and Phe-Pro (280 Da). The proportional elution volume (K_{av}) is defined as $(V_e - V_o)/(V_t - V_o)$ where V_e is the elution volume of the peptide, V_o is the void volume and V_t is the total volume. V_o and V_t of the column were experimentally determined as 8.8 and 20.5 mL, respectively.

3. Results and discussion

The time course of GLP-1 and GLP-2 degradation by DPP IV is shown in Fig. 1. The DPP IV activity used (25 unit/L) is similar to the normal level in human plasma. With this particular enzyme preparation, the degradation of GLP-1 is slower than reported for human serum and truncation of GLP-2 is faster than the published half-life of 3–8 hr in human plasma or whole blood [4,10,19]. The *in vitro* kinetic data reported here correspond with the *in vivo* observation that intact GLP-1 disappears faster than GLP-2. In the conditions of Fig. 1, GLP-1 disappears nine times faster than GLP-2. The result signifies that when DPP IV encounters both peptides in equal concentrations GLP-1 will be preferentially truncated. Fitting the data on concentration dependency with the Michaelis–Menten equation yields a K_m of 36 μM (SE = 7) and a k_{cat} of 7.1 s^{-1} (SE = 0.5) for GLP-1. The K_m for GLP-2 is very similar, 36 μM (SE = 6), so that the major difference resides in the k_{cat} value of 0.87 s^{-1} (SE = 0.06; SE is the standard error of the fit).

The peptide concentrations required for the determination of kinetic parameters typically are orders of magnitude higher than those required for biological activity. GLP-1 and several other DPP IV substrates (e.g. chemokines) tend to form aggregates at high (μM to mM) concentrations. In order to verify whether aggregate formation could have influenced our kinetic data, gel filtration experiments were carried out. We used a high-resolution size exclusion column optimized for a molecular weight range of

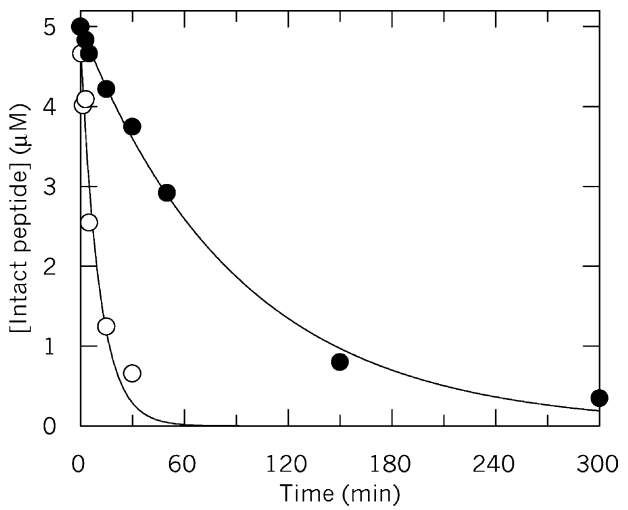

Fig. 1. Time course of truncation of 5 μM GLP-1 (○) and GLP-2 (●) by 25 unit/L DPP IV at 37° (from three independent incubations for each peptide).

```
GLP-1   HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG
GLP-2   HADGSFSDEMNTILDNLAARDFINWLIQTKITD
            ttthhhhhhhttthhhhthhhhs
```

Fig. 2. Aligned sequences of GLP-1 and GLP-2 show differences in the amino acids following the scissile bond and interspersed among the entire peptide length. Residues in α-helices and turns are marked h and t, respectively (based upon the X-ray structure of glucagon [27]).

100–7000. In the buffer of the kinetic experiments GLP-1 (MW = 3298) eluted with a K_{av} of 0.35 and GLP-2 (MW = 3922) with a K_{av} of 0.27. In a separate experiment, 0.1% TFA and 30% acetonitrile were added to the sample and the elution buffer to minimize both ionic and hydrophobic interactions between the peptides and the column matrix. In these conditions both GLP-1 and GLP-2 eluted with a proportional elution volume of 0.26, just before GRP(3–27) used as a calibrator (MW = 2660, K_{av} = 0.29). Although GLP-1 eluted somewhat later than expected (in assay buffer), these results revealed that neither GLP-1 nor GLP-2 form aggregates under the conditions used in the kinetic experiments. The effect on k_{cat} must, therefore, originate from differences in the GLP-1 and GLP-2 sequence or structure which seem in this case more important for transition state stabilization than for the formation of the enzyme–substrate complex. The sequence of GLP-1 and GLP-2 is shown in Fig. 2. GLP-1, glucagon and related peptides adopt a mainly α-helical conformation, with considerable flexibility of the N-terminal amino acids [26,27]. Since the N-terminal amino acids of GLP-1 and GLP-2 are highly conserved, it may be assumed that the interactions important for the selectivity involve nonconserved residues distributed along the entire length of the peptides. Several observations with DPP IV substrates support the importance of long range interactions for substrate binding and catalysis [23,24,28]. Dissociation constants of 10–100 μM for GLP-1 and its truncated form were determined in biosensor experiments independently from the DPP IV catalytic activity [28].

We compared the specificity constants (k_{cat}/K_m) of the glucagon-like peptides with other peptide substrates that have been studied with DPP IV from the same source and in an identical experimental set-up (Fig. 3). GLP-1 clearly is a good substrate, with a k_{cat}/K_m value comparable to some of the chemokines which are cleaved after a proline. The generally accepted selectivity of DPP IV for proline and alanine is less pronounced in this collection of natural peptides. The specificity constant of the second cleavage of MDC (after Gly^4) is even slightly higher than the truncation after Ala^2 in GLP-1. In PACAP 38, the cleavage after Ser^2 is as efficient as after Ala^2 in GLP-2. The molecular origin of the difference is not yet fully understood.

In conclusion, this study contributes to the biochemical characterization of glucagon-like peptide metabolism. To the best of our knowledge, this is the first report on the *in vitro* kinetic parameters of DPP IV mediated GLP-2 truncation. The situating of both glucagon-like peptides among other natural substrates of DPP IV might provide a better insight in the *in vivo* effects of DPP IV inhibitors.

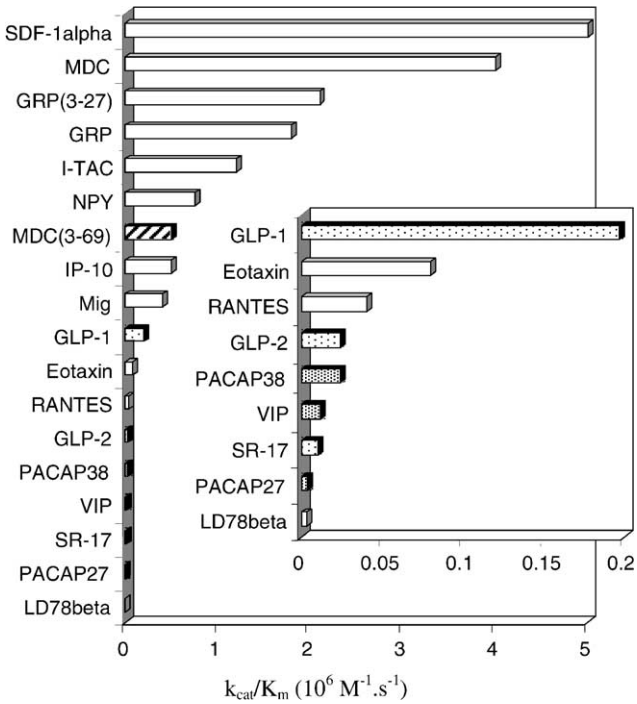

Fig. 3. Comparison of the specificity constants (k_{cat}/K_m) for natural peptides, studied in identical experimental conditions [23,24,29]. The lower part of the graph is expanded (inset) to highlight the difference between GLP-1 and GLP-2. The graph provides the relative position of each peptide within a 1000-fold range of specificity constants. The experimental errors of the specificity constants vary between 5 and 20% depending on the peptide. The pattern of the bars represents the type of N-terminal penultimate residue: proline (open), alanine (light gray dotted), glycine (striped), serine (dark gray dotted).

Acknowledgments

The work presented is supported by grants of the Fund for Scientific Research Flanders and of the University of Antwerp (BOF project).

References

[1] Dhavantari S, Seidah NG, Brubaker PL. Role of prohormone convertases in the tissue-specific processing of proglucagon. Mol Endocrinol 1996;10:342–55.
[2] Drucker DJ. Biological actions and therapeutic potential of the glucagon-like peptides. Gasteroenterology 2002;122:531–44.
[3] Drucker DJ. Gut adaptation and the glucagon-like peptides. Gut 2002;50:428–35.
[4] Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide 1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 1993;214:829–35.
[5] Kieffer TJ, McIntosh CHS, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like

peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 1995;136:3585–96.

[6] Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously an intraveneously administered glucagon-like peptide 1 are rapidly degraded from the NH_2-terminus in type II diabetic patients and healthy subjects. Diabetes 1995;44: 1126–31.

[7] Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7–36) amide is transformed to glucagon-like peptide-1-(9–36) amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 1999;140:5356–63.

[8] Brubaker PL, Crivici A, Izzo A, Ehrlich P, Tsai C-H, Drucker DJ. Circulating and tissue forms of the intestinal growth factor, glucagon-like peptide 2. Endocrinology 1997;138:4837–43.

[9] Drucker DJ, Shi Q, Sumner-Smith M, Tavares W, Hill M, Deforest L, Cooper S, Brubaker PL. Regulation of the biological activity of glucagon-like peptide 2 by dipeptidyl peptidase IV. Nat Biotechnol 1997;15:673–7.

[10] Hartmann B, Harr MB, Jeppesen PB, Wojdemann M, Deacon CF, Mortensen PB, Holst JJ. In vivo and in vitro degradation of glucagon-like peptide-2 in humans. J Clin Endocrin Metab 2000;85:2884–8.

[11] Deacon CF, Plamboeck A, Møller S, Holst JJ. GLP-1-(9–6) amide reduces blood glucose in anesthetized pigs by a mechanism that does not involve insulin secretion. Am J Physiol Endocrinol Metab 2002;282:E873–7.

[12] Dacambra MP, Yusta B, Sumner-Smith M, Crivici A, Drucker DJ, Brubaker PL. Structural determinants for activity of glucagon-like peptide-2. Biochemistry 2000;39:8888–94.

[13] Thulesen J, Knudsen LB, Hartmann B, Hastrup S, Kissow H, Jeppesen PB, Orskov C, Holst JJ, Poulsen SS. The truncated metabolite GLP-2 (3–33) interacts with the GLP-2 receptor as a partial agonist. Regul Pept 2002;103:9–15.

[14] Holst JJ, Deacon CF. Inhibition of the activity of dipeptidyl peptidase IV as a treatment for type 2 diabetes. Diabetes 1998;47:1663–70.

[15] Hartmann B, Thulesen J, Kissow H, Thulesen S, Orskov C, Ropke C, Poulsen SS, Holst JJ. Dipeptidyl peptidase IV inhibition enhances the intestinotrophic effect of glucagon-like peptide-2 in rats and mice. Endocrinology 2000;141:4013–20.

[16] Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, McIntosh CH, Pederson RA. Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats. Diabetes 2002;51:943–50.

[17] Ahrén B, Simonsson E, Larsson H, Landin-Olsson M, Jansson P-A, Sandqvist M, Båvenholm P, Efendic S, Eriksson JW, Dickinson S, Holmes D. Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. Diabetes Care 2002;25:869–75.

[18] Reimer MK, Holst JJ, Ahrén B. Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. Eur J Endocrinol 2002;146:717–27.

[19] Pauly RP, Rosche F, Wermann M, McIntosh CH, Pederson RA, Demuth HU. Investigation of glucose-dependent insulinotropic polypeptide (1–42) and glucagon-like peptide-1-(7–36) degradation in vitro by dipeptidyl peptidase IV using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry. A novel kinetic approach. J Biol Chem 1996;271:23222–9.

[20] Mentlein R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regul Pept 1999;85:9–24.

[21] De Meester I, Korom S, Van Damme J, Scharpé S. CD26 let it cut or cut it down. Immunol Today 1999;20:367–75.

[22] De Meester I, Durinx C, Proost P, Scharpé S, Lambeir A-M. DPIV—natural substrates of medical importance. In: Langner J, Ansorge S, editors. Ectopeptidases CD13/aminopeptidase-*N* and CD26/dipeptidylpeptidase IV in medicine and biology. New York: Kluwer, Academic/Plenum Publishers, 2002. p. 223–57.

[23] Lambeir A-M, Proost P, Durinx C, Bal G, Senten K, Augustyns K, Scharpé S, Van Damme J, De Meester I. Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. J Biol Chem 2001;276:29839–45.

[24] Lambeir A-M, Durinx C, Proost P, Van Damme J, Scharpé S, De Meester I. Kinetic study of the processing by dipeptidyl peptidase IV/CD26 of neuropeptides involved in pancreatic insulin secretion. FEBS Lett 2001;507:327–30.

[25] De Meester I, Vanhoof G, Lambeir A-M, Scharpé S. Use of immobilized adenosine deaminase (EC 3.5.4.4) for the rapid purification of native human CD26/dipeptidyl peptidase IV (EC 3.4.14.5). J Immunol Methods 1996;189:99–105.

[26] Chang X, Keller D, O'Donoghue S, Led JJ. NMR studies of the aggregation of glucagon-like peptide-1: formation of a symmetric helical dimer. FEBS Lett 2002;515:165–70.

[27] Sasaki K, Dockerill S, Adamiak DA, Tickle IJ, Blundell T. X-ray analysis of glucagon and its relationship to receptor binding. Nature 1975;257:751.

[28] Hinke SA, Kühn-Wache K, Hoffmann T, Pederson RA, McIntosh CHS, Demuth H-U. Metformin effects on dipeptidyl peptidase IV degradation of glucagon-like peptide-1. Biochem Biophys Res Commun 2002;291:1302–8.

[29] Depreitere J, Durinx C, Wang Z, Coen E, Lambeir A-M, Scharpé S, De Potter W, Nouwen EJ. Presence and release of SR-17 (chromogranin $B_{586–602}$) in the porcine splenic nerve and its enzymatic degradation by CD26/dipeptidyl peptidase IV. Regul Pept 2002;106:17–9.