Prospects for the management of type 2 diabetes using food protein-derived peptides with dipeptidyl peptidase IV (DPP-IV) inhibitory activity

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Abstract

Since drug based inhibition of dipeptidyl peptidase IV (DPP-IV) is employed in type 2 (T2D) diabetes therapy, food protein hydrolysates which inhibit DPP-IV may also have potential in the management of T2D. Specific peptide motifs, consisting of an N-terminal Trp and/or a Pro at position 2, have been associated with relatively potent inhibition of DPP-IV. Different modes of inhibition which may, or may not, involve the active site of DPP-IV have been identified. Animal studies have shown that food protein hydrolysates having *in vitro* DPP-IV inhibitory activity generally yield antidiabetic effects *in vivo*. However, clear evidence of such effects in humans is still required in order to establish the potential role of food protein hydrolysates in the management of T2D.

Introduction

Type 2 diabetes (T2D) is a major component of the metabolic syndrome. It has been shown to affect a growing number of people worldwide. While the etiology of T2D is not fully understood a link with obesity or high abdominal body fat content has been proposed. The role of food proteins in the regulation of serum glucose in humans has been demonstrated in several human intervention studies [for reviews, see: 1, 2, 3]. Human intervention studies with food proteins and food protein hydrolysates have involved a wide range of dietary proteins originating from animal and marine as well as plant sources [4-6]. However, the mechanism(s) of action explaining the antidiabetic effects observed are currently not fully understood. It is thought that dietary amino acids and short peptides may impact in a number of ways including: (a) the direct stimulation of pancreatic cells leading to increased insulin secretion, (b) inhibition of metabolic enzymes involved in the regulation of serum glucose, such as dipeptidyl peptidase IV (DPP-IV) and α-glucosidase, and (c) secretion of incretins (i.e., glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)).

DPP-IV is an ubiquitous enzyme which has been shown to cleave and inactivate GLP-1 and GIP in the post-prandial phase, leading to a loss in their insulinotropic activity [7]. DPP-IV inhibition is currently a key target in the treatment of T2D. In this context, different DPP-IV inhibitory drugs, belonging to a class known as gliptins, have been developed and marketed [8]. Gliptins generally have a high potency, with a half maximal inhibitory concentration (IC_{50}) in the nM range. Interestingly, over the past 30 years, different naturally-derived peptides have been shown to inhibit DPP-IV. DPP-IV inhibitory peptide sequences have notably been identified within food proteins. The DPP-IV inhibitory properties of food protein hydrolysates and associated peptides have recently been reviewed [1, 3, 9, 10]. To date, the most potent DPP-IV inhibitory peptide is Ile-Pro-Ile (diprotin A), which was originally identified in *Bacillus cereus* culture filtrates [11]. Ile-Pro-Ile is also present in several dietary proteins such as bovine κ-casein, chicken egg

ovotransferrin and the phycoerythrin β subunit from the macroalga *Palmaria palmata* [12].

The aim of this review was to assess the current literature in respect to food protein hydrolysates/peptides and their DPP-IV inhibitory properties. The link between DPP-IV inhibition and antidiabetic effects was also assessed with the view of determining the potential of food protein hydrolysates for the management of T2D.

Potential food protein sources of DPP-IV inhibitory peptides

Different dietary proteins have been identified as a source of DPP-IV inhibitory peptides using *in silico* approaches. *In silico* approaches have focused on researching previously identified DPP-IV inhibitory peptide sequences within various food proteins. The outcomes of these studies indicate that milk proteins are particularly rich in DPP-IV inhibitory peptide motifs [12, 13]. The limitations of *in silico* approaches reside in the necessity to subsequently develop a strategy (i.e., enzymatic hydrolysis or fermentation) to release the target peptides from the protein.

To date, most of the *in vitro* studies appear to have used enzymatic hydrolysis of food proteins to release DPP-IV inhibitory peptides [14-18]. There are a limited number of studies which demonstrate that microbial fermentation could also be utilized for the generation of DPP-IV inhibitory peptides. Water soluble extracts from cheese, for example, have been identified for their DPP-IV inhibitory properties [19]. Food protein hydrolysis is typically conducted in aqueous media using commercially available food-grade enzyme preparations which are added at a known enzyme to substrate ratio. Several studies have described the utilization of gastrointestinal (e.g., pepsin, trypsin, Pancreatin, Corolase PP), plant (e.g., bromelain and papain) or microbial (e.g., Alcalase, Flavourzyme and Protamex) [14, 15, 18, 20-24] enzyme preparations to generate food protein hydrolysates with DPP-IV inhibitory properties. The hydrolysis conditions (i.e., pH and temperature) are generally chosen to correspond to the optimum conditions for the enzyme activity employed with hydrolysis durations of up to several hours to ensure the release of bioactive peptides. Further fractionation of food protein hydrolysates, using

techniques such as ultrafiltration, solid phase extraction and chromatographic (reverse-phase, cation-exchange, size-exclusion and thin layer) separations, have been used to obtain fractions enriched in more potent DPP-IV inhibitory peptides [16, 18, 25-29].

Generally, the percentage of DPP-IV inhibition is assessed following incubation of DPP-IV with the peptides/hydrolysates in the presence of a chromogenic substrate (e.g., Gly-Pro-p-nitroanilide (pNA), Gly-Pro-aminomethylcoumarin (AMC) or Gly-Pro-aminoluciferin). Various *in vitro* protocols, which may vary in terms of the origin of DPP-IV (human recombinant vs. animal extract), nature of the DPP-IV substrate, enzyme to substrate ratio, duration of incubation and pH, have been described in the scientific literature to assess the DPP-IV inhibitory potential of food protein-derived peptides [12]. These differences in experimental conditions may explain, in certain instances, the variations observed for the potency of selected peptide sequences [30]. While most of the *in vitro* evaluation of DPP-IV inhibitory properties of food protein hydrolysates has been conducted with milk proteins [14, 15, 19, 31-34], alternative protein substrates from meat/animal skin [35-38], marine [18, 24, 39-41] and plant [17, 20-23, 42-45] origin have also been described in the literature. To date, the most potent *in vitro* DPP-IV inhibitory food protein hydrolysates have been reported for a peptic digest of bovine α-lactalbumin with an IC_{50} of 0.036 mg mL^{-1} [31] and a simulated gastro-intestinal digest (pepsin/Pancreatin) of Navy beans having an IC_{50} of 0.093 mg mL^{-1} [43]. Differences in DPP-IV inhibition potency between food protein hydrolysates may generally be explained by their unique peptide composition but may also to some extent be dependent on the assay employed [46].

Structure-function of DPP-IV inhibitors

Research on DPP-IV inhibitory peptides from food protein sources is relatively novel (< 10 years). Therefore, the number of peptide sequences which have been identified to date is limited (< 100 peptide sequences) [12, 33].

Elucidation of the physicochemical characteristics of peptides which are linked to DPP-IV

inhibition has been attempted. To date, there does not seem to be a consensus for the physicochemical characteristics of peptides which display relatively potent DPP-IV inhibition [47]. However, using a peptide alignment strategy, it has been shown that peptides containing a Trp at the N-terminus and a Pro at position 2 were generally relatively potent DPP-IV inhibitors, having IC_{50} values < 200 µM [12].

Several novel peptide sequences have been identified within food protein hydrolysates using liquid chromatography mass-spectrometric (LC-MS) analyses generally coupled with bioactivity-driven fractionation approaches [16, 17, 19, 25, 29, 48]. *In silico* approaches have also allowed the identification of numerous peptide sequences [14, 34, 49, 50]. In addition, systematic approaches based on peptide library [51-53] and peptide array [48] technologies have enhanced DPP-IV inhibitory peptide sequence discovery as they allow rapid screening of hundreds of peptides.

Mode of action of dietary DPP-IV inhibitory peptides

Different modes of action of DPP-IV inhibitory peptides have been reported. These include competitive, non-competitive, uncompetitive and mixed-type inhibition [14, 17, 27, 50]. Knowledge of the mode of action of DPP-IV inhibitory peptides is important in order to understand their site of interaction with DPP-IV. This information is relevant when studying the molecular docking of peptides to the active site of DPP-IV [47].

In addition to the different modes of inhibition, it has been shown that specific peptides could act as DPP-IV substrates. Well-known examples of substrate inhibitors of DPP-IV are Ile-Pro-Ile and Val-Pro-Ala [54]. Food protein-derived peptides which are susceptible to DPP-IV cleavage have been classified as substrate or prodrug type inhibitors. Both substrate and prodrug peptide inhibitors comprise the typical motifs of DPP-IV substrates, i.e., Xaa-Pro- or Xaa-Ala- (where Xaa is an amino acid), at their N terminus. The cleavage of substrate inhibitors generally induces a loss/reduction in their bioactive properties. In contrast, in the case of prodrug inhibitors, DPP-

IV releases a more potent peptide. Interestingly, DPP-IV substrates have been predicted *in silico* to be released by the action of gastrointestinal enzymes on milk proteins [55]. In particular, a prodrug inhibitor, Leu-Pro-Leu-Pro-Leu (β-casein (f 135-139), IC_{50} = 325 μM), was shown to be cleaved by DPP-IV *in vitro*, releasing a more potent compound Leu-Pro-Leu (IC_{50} = 241 μM). Therefore, the susceptibility of selected peptides to DPP-IV cleavage may have consequences *in vivo*, resulting in either a loss or an increase in their bioactive properties.

In vitro studies have evaluated the effect of combining the DPP-IV inhibitory drug Sitagliptin with DPP-IV inhibitory peptides and a whey protein hydrolysate [26]. Using binary mixtures of Sitagliptin and dipeptide, together with an isobole approach, an additive effect on DPP-IV inhibition was shown in most instances [56]. Furthermore, synergistic effects were observed with Ile-Pro-Ile-Gln-Tyr (κ-casein (f 26-30)). While these effects have been observed *in vitro*, they need to be evaluated *in vivo* in order to determine if it is possible to combine drugs and food protein hydrolysates to, for example, restrict the possible side-effects associated with antidiabetic medicines [for review, see: 1].

Evidence of antidiabetic effects of food-protein derived DPP-IV inhibitory peptides *in vivo*

The *in vivo* studies reporting the antidiabetic effects of DPP-IV inhibitory food protein hydrolysates, conducted to date, have been carried out in small animals. To our knowledge, six animal studies have been carried out to date with zein and meat protein hydrolysates [44], milk protein-derived peptides and hydrolysates [19, 32] along with gelatin hydrolysates from Atlantic salmon [39] halibut and tilapia [57] as well as porcine skin [36]. The outcomes of these studies are summarized in Table 1. All studies demonstrated a reduction in glycaemia. This was linked, only in certain instances, to an increase in post-prandial insulin level following ingestion of the hydrolysates [36, 39, 44, 57]. In addition, four animal studies have also demonstrated a reduction

in plasma DPP-IV activity, which was associated with an increase in the plasma level of active and/or total GLP-1 [36, 39, 44, 57].

To date, the study of DPP-IV inhibition by dietary peptides in humans is in its infancy. A number of studies have analyzed serum DPP-IV activity following nutritional interventions. However, to our knowledge, none of these studies have demonstrated a reduction in DPP-IV activity as a consequence of food-protein or hydrolysate consumption [58]. Interestingly, several fragments from bovine β-casein have been reported in the gastrointestinal tract of humans [59], some of which had previously been described for their *in vitro* DPP-IV inhibitory properties [60] (Table 2). However, to date, clear evidence for the bioavailability of food protein-derived peptides is limited [2], making it challenging to study their effects on systemic targets. This reinforces the relevance of targeting DPP-IV inhibition directly in the gut as opposed to the serum or other organs. To our knowledge, no study to date has evaluated DPP-IV inhibition directly in the gut of animals or humans in the context of nutritional interventions.

Conclusions and perspectives

To date, dietary protein hydrolysates with DPP-IV inhibitory properties have mainly been studied *in vitro*. A limited number of studies have been performed *in vivo*, with the majority of the studies being conducted in small animals. The contribution of DPP-IV inhibition to serum glucose regulatory effects following dietary protein and hydrolysate ingestion by humans is still unknown. However, it is likely that DPP-IV inhibition may play a role in the antidiabetic effects of intact and hydrolysed food proteins in humans.

Analysis of the current literature has allowed identification of several opportunities to further study the DPP-IV inhibitory potential of food protein hydrolysates. There is a requirement for human intervention studies to better understand the role of DPP-IV inhibitory peptides in serum glucose regulation. The interactive effects between food protein-derived peptides *in vivo* and antidiabetic drugs is also worthy for future studies. Additional studies on the interaction of

peptides with secondary binding sites of DPP-IV are warranted as numerous non-competitive peptide sequences have been found to be relatively potent inhibitors of DPP-IV. Finally, utilization of *in silico* approaches may help in the identification of novel food protein sources of DPP-IV inhibitory peptides. This may allow valorization of underutilized proteins as well as the development of strategies for the release of potent DPP-IV inhibitory peptides.

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Conflicts of interests

The authors declare that they have no conflict of interest.

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This review presents the scientific information linking the ingestion of intact milk proteins to a range of bioactive properties in humans.

Table captions

Table 1. Summary of the outcomes of animal studies conducted with food protein-derived hydrolysates displaying dipeptidyl peptidase IV (DPP-IV) inhibitory properties.

Table 2. Peptides originating from bovine β-casein identified in the jejunum of human subjects which display *in vitro* dipeptidyl peptidase IV (DPP-IV) inhibitory activity. Adapted from Boutrou *et al.* [59].

Table 1

Compound	Study design	Biological outcomes	Reference
Zein (ZH) and meat protein hydrolysates (MPH)	Animals: 7 week old ♂ Sprague-Dawley rats (n=6/group) Dose: 2 g kg^{-1} direct ileal administration Duration: acute	Antidiabetic effects observed with ZH only but not MPH. - Increased total & active GLP-1 secretion from L cells - Reduced plasma DPP-IV activity - Increased insulin secretion - Reduced glycaemia	[44]
β-Lactoglobulin hydrolysed with trypsin	Animals: C57BL/6 mice (n=10/group) Dose: 300 mg kg^{-1} oral gavage Duration: acute	- Reduced glycaemia following an OGTT	[32]
LPQNIPPL[a] (β-casein (f 70-77))	Animals: 8 week old ♀ Sprague-Dawley (n=12/group) Dose: 300 mg kg^{-1} oral gavage Duration: acute	- Reduced glycaemia following an OGTT - No effect on plasma insulin levels	[19]
Porcine skin gelatin hydrolysed with Flavourzyme™	Animals:8 weeks ♂ Sprague-Dawley streptozotocin (STZ)-induced diabetic rats (n=12/group) Dose: 300 mg day^{-1} oral gavage Duration: 42 days	- Increased active plasma GLP-1 secretion - Reduced plasma DPP-IV activity - Increased plasma insulin levels - Increased plasma glucagon levels - Reduced glycaemia following an OGTT	[36]
Atlantic salmon skin gelatin hydrolysed with Flavourzyme™	Animals: ♂ Sprague-Dawley STZ-induced diabetic rats (n=12/group) Dose: 300 mg day^{-1} oral gavage Duration: 5 weeks	- Increased total & active plasma GLP-1 secretion - Reduced plasma DPP-IV activity - Increased plasma insulin levels - Increased insulin:glucagon ratio - Reduced glycaemia following an OGTT	[39]

Tilapia (TSGH) and halibut (HSGH) skin gelatin hydrolysed with Flavourzyme™	Animals: ♂ Sprague-Dawley STZ-induced diabetic rats (n=11/group) Dose: 750 mg kg^{-1} day^{-1} oral gavage Duration: 30 days	- Increased total plasma GLP-1 secretion with TSGH and HSGH - Reduced plasma DPP-IV activity with TSGH and not HSGH - Increased plasma insulin levels, being more marked with TSGH than with HSGH - Reduced glycaemia following an OGTT, being more marked with TSGH than with HSGH	[57]

[a]peptide sequence with the one letter amino acid code.

DPP-IV: dipeptidyl peptidase IV; GLP-1: glucagon like peptide 1; HSGH: halibut skin gelatin hydrolysate; MPH: meat protein hydrolysate; OGTT: oral glucose tolerance test; STZ: streptozotocin; TSGH: tilapia skin gelatin hydrolysate; ZH: zein hydrolysate; ♀: female; ♂: male.

Table 2

Peptide fragment	Compound[a]	DPP-IV IC_{50} value (μM)[b]	Reference[c]
60-68	YPFPGPIPN	670	[19]
62-68	FPGPIPN	260	[19]
70-77	LPQNIPPL	46	[19]
71-77	PQNIPPL	1500	[19]
74-82	IPPLTQTPV	1300	[19]
135-139	LPLPL	325	[50]
-	Sitagliptin	39×10^{-3}	[56]

[a]The peptide sequences are abbreviated with the one letter amino acid code
[b]IC_{50}: half maximal inhibitory concentration
[c]Bibliographic reference reporting the *in vitro* IC_{50} value