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Inhibition of Dipeptidyl Peptidase (DPP)-IV and α-Glucosidase Activities by Pepsin-Treated Whey Proteins

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ABSTRACT: Inhibitors of the enzymes dipeptidyl peptidase (DPP)-IV and α-glucosidase are two classes of pharmacotherapeutic agents used for the treatment of type 2 diabetes. In the present study, whey protein isolate (WPI), α-lactalbumin, β-lactoglobulin, serum albumin, and lactoferrin hydrolysates obtained by peptic digestion were investigated for their potential to serve as natural sources of DPP-IV and α-glucosidase inhibitors. Although inhibition of DPP-IV activity was observed in all pepsin-treated whey proteins studied, the α-lactalbumin hydrolysate showed the greatest potency with an IC_{50} value of 0.036 mg/mL. Conversely, only WPI, β-lactoglobulin, and α-lactalbumin hydrolysates displayed some inhibitory activity against α-glucosidase. This study suggests that peptides generated from whey proteins may have dual beneficial effects on glycemia regulation and could be used as functional food ingredients for the management of type 2 diabetes.

KEYWORDS: *α-glucosidase inhibitor, dipeptidyl peptidase (DPP)-IV inhibitor, peptide, type 2 diabetes, whey protein*

INTRODUCTION

Type 2 diabetes represents one of the most challenging health problems of the 21st century. Both the prevalence and incidence of this chronic and multifactorial disorder, which is characterized by defects in insulin secretion and/or action,[1] are increasing at an alarming rate worldwide.[2] When not properly treated, type 2 diabetes can lead to serious complications, including cardiovascular disorders, blindness, and renal failure.[3] Therefore, effective diabetes management strategies are of primary importance.

Eleven classes of glucose-lowering agents are currently available for the management of diabetes. Of these, dipeptidyl peptidase-IV (DPP-IV; EC 3.4.14.5) inhibitors are among the newest medications that have been introduced to the type 2 diabetes pharmacopeia.[4,5] These orally available agents exert their beneficial effect on glycemia regulation by enhancing the endogenous concentrations of the active incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1).[6,7] The incretins are gut-derived hormones that stimulate pancreatic glucose-dependent insulin secretion, suppress pancreatic glucagon release, delay gastric emptying, and modulate appetite.[8−10] Both GIP and GLP-1 are endogenous physiological substrates of DPP-IV and are rapidly inactivated by the enzyme.[11] A number of synthetic DPP-IV inhibitors have been developed over the past few years, including the peptide-derived peptidomimetic inhibitors Galvus (vildagliptin) from Novartis and Onglyza (saxagliptin) from Bristol-Meyers Squibb as well as the nonpeptidomimetic inhibitors Januvia (sitagliptin) from Merck, Nesina (Alogliptin) from Takeda, and Tradjenta (Linagliptin) from Boehringer Ingelheim. These drugs typically cause a 2-fold increase in endogenous GLP-1 levels and have shown promising results in the management of type 2 diabetes.[8]

More recently, studies in the area of bioactive peptides have suggested that dietary proteins could be natural precursors of DPP-IV inhibitors. An in silico analysis revealed that peptide sequences matching those reported in the literature to present inhibitory activity against DPP-IV are contained within the primary sequences of various proteins from plant and animal sources, including dairy and fish proteins.[12] Moreover, hydrolysates and peptides from a number of proteins, including those from egg,[13] fish,[14,15] amaranth,[16] rice bran,[17] corn,[18] and milk,[19−24] have been found to be able to inhibit the activity of DPP-IV. Among those proteins, dairy proteins seem to be a particularly interesting source of DPP-IV inhibitors, peptides derived from the whey and casein fractions of milk having been shown to display DPP-IV inhibitory activity in vitro[19−24] and to reduce blood glucose levels in vivo.[21,22] Although β-casein[22] and β-lactoglobulin[19−21] have been investigated for their DPP-IV inhibitory activity, little is known on the potential of other individual milk proteins to serve as precursors of DPP-IV inhibitory peptides.

Another therapeutic approach in the management of type 2 diabetes consists in the suppression of the digestion/absorption of glucose in the gut by the inhibition of carbohydrate-hydrolyzing enzymes such as α-glucosidase.[25] In the past decade, numerous studies have been carried out to identify dietary products or constituents that may serve as natural sources of inhibitors of these enzymes. A variety of fruits and vegetables, including blueberry,[26] strawberry,[26,27] broccoli sprouts,[28] and green pepper,[28] have been shown to display α-amylase and/or α-glucosidase inhibitory activity. Polyphenolic compounds, such as flavonoids and tannins, in these foods have been suggested to be responsible for the observed inhibitory activity.[26,29−32] In addition to food commodities of plant origin, peptides derived from sardine muscle[33] and more recently egg white protein[34] have also been reported to suppress the activity

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of α-glucosidase. To our knowledge, the α-glucosidase inhibitory activity of dairy protein hydrolysates has not been reported.

The consumption of dairy proteins, particularly whey protein, has been shown in a number of intervention studies to have beneficial effects on glucose metabolism.[35,36] The addition of whey protein to high carbohydrate containing meals has been found to lead to a reduction in postprandial blood glucose levels and an increase in plasma insulin concentrations in both healthy and type 2 diabetes individuals.[35,36] The antidiabetic properties of whey protein have been suggested to be attributable to its content of bioactive peptides which, following their release during gastrointestinal digestion, could stimulate the secretion of gut-derived hormones and/or inhibit enzymes involved in glycemia homeostasis.[35,36] Little is known, however, on the specific whey proteins from which these peptides originate.

In a previous investigation to assess whether inhibition of the DPP-IV enzyme may be one of the potential mechanisms contributing to the reported benefits of whey protein on regulating glucose metabolism, we found that, among several enzymatic treatments using various proteases, the peptic digestion of whey protein isolate (WPI) generated the hydrolysate with the greatest DPP-IV inhibitory activity.[23] The specific protein constituents in the peptic WPI hydrolysate responsible for the observed inhibitory activity were, however, not identified. Also unknown is the ability of whey protein hydrolysates to inhibit other enzymes, such as α-glucosidase, which are involved in glycemia regulation. Thus, the objective of the present study was to investigate the DPP-IV and α-glucosidase inhibitory activities of protein hydrolysates obtained by peptic digestion of WPI and the four major protein constituents of whey, namely, β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), and lactoferrin (Lf).

MATERIALS AND METHODS

Materials. Pepsin (EC 3.4.23.1, from porcine gastric mucosa, ≥2500 units per mg protein), dipeptidyl peptidase-IV (DPP-IV, EC 3.4.14.5, from porcine kidney, ≥10 units per mg protein), α-glucosidase in the form of rat intestine acetone powder, acarbose, maltose, Ile-Pro-Ile (diprotin A), glucose assay kit (*o*-dianisidine reagent and glucose oxidase/peroxidase reagent), α-lactalbumin from bovine milk (≥85% protein), albumin from bovine serum (≥96% protein), and β-lactoglobulin from bovine milk (≥90% protein) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Trinitrobenzenesulfonic acid (TNBS, in methanolic solution) was from Thermo Scientific (Rockford, IL, USA). Whey protein isolate (WPI 895, Fonterra, NZ; 92.4% protein) was donated by Nealanders International Inc. (Delta, BC, Canada), and lactoferrin from bovine milk was supplied by DMV International (Fraser, NY, USA; 80% protein). Gly-Pro-*p*-nitroanilide (H-Gly-Pro-*p*NA·HCl) was from Bachem Americas (Torrance, CA, USA). Ultralow Range Molecular Weight Marker (1.0−26.6 kDa) was from Sigma-Aldrich (Oakville, ON, Canada), whereas BioReagents EZ-Run *Rec* Protein Ladder (10−200 kDa) was from Fisher Scientific (Fair Lawn, NJ, USA). Polyacrylamide precast gels (gradient 10−15 and high density), PhastGel SDS buffer strips, PhastGel Blue R tablets, and PlusOne silver staining kit were purchased from GE Healthcare (Uppsala, Sweden).

Peptic Hydrolysis of Whey Proteins. Whey protein isolate (WPI), α-lactalbumin, β-lactoglobulin, lactoferrin, and bovine serum albumin solutions (3% protein, w/v, in deionized distilled (dd) H_2O containing 0.02% sodium azide) were adjusted to pH 2.0 using 6 M HCl and preincubated in a water bath with shaker (Blue M Electric Co., Blue Island, IL, USA) to bring the temperature to 37 °C. The pepsin enzyme was added (4% enzyme/substrate ratio on a w/w protein basis), and the solutions were incubated at 37 °C under constant agitation for 60 min. The hydrolyzed whey proteins were then centrifuged using a DuPont Sorvall Centrifuge RD 5B (Mandel Scientific Co. Ltd., Guelph, ON, Canada) at room temperature for 10 min at 12100*g*. The supernatants were collected, freeze-dried, and stored at −18 °C until further analysis.

Extent of Hydrolysis. The extent of hydrolysis of the hydrolyzed whey protein samples was assessed by the determination of the content of free α-amino groups measured in triplicate by the TNBS reaction as described by Lacroix and Li-Chan.[23] The α-amino group content was expressed in millimoles per gram protein by reference to an L-leucine standard curve.

SDS-PAGE. The unhydrolyzed and hydrolyzed whey proteins were characterized by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE). The samples (80 μL of 0.1−1 mg/mL solutions in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) were combined with 20 μL of 10% SDS, 2 μL of 2-mercaptoethanol, and 1 μL of bromophenol blue (1%, w/v), to yield final concentrations of 0.078 mg/mL for the unhydrolyzed whey protein samples, 0.39 mg/mL for whey protein isolate and β-lactoglobulin hydrolysates, and 0.78 mg/mL for lactoferrin, α-lactalbumin, and bovine serum albumin hydrolysates. The mixtures were submerged in boiling water for 20 min and centrifuged at 14000 rpm for 5 min using a VWR Galaxy 16 microcentrifuge (VWR Scientific Products, Bristol, CT, USA), and 1 μL of the supernatants was loaded onto the gels. The electrophoresis was performed on a PhastSystem (Pharmacia Biotech, Uppsala, Sweden) using PhastGel gradient 10−15 and PhastGel high-density precast gels for the unhydrolyzed proteins and hydrolysates, respectively. The unhydrolyzed whey proteins were revealed by silver staining, whereas the hydrolysate samples were detected using Coomassie blue stain followed by silver stain. Stainings were performed according to the manufacturer's instructions. Molecular weight markers ranging from 10 to 200 kDa and from 1.0 to 26.6 kDa were run with the unhydrolyzed and hydrolyzed samples, respectively.

Determination of DPP-IV Inhibitory Activity. The effect of the hydrolyzed whey protein samples on DPP-IV activity was determined using a modified version of the DPP-IV assay described by Lacroix and Li-Chan.[23] The samples were reconstituted in 100 mM Tris-HCl buffer, pH 8.0, to concentrations ranging from 0.0125 to 1.25 mg/mL (final assay concentrations). In a 96-well microplate, 25 μL of test sample was preincubated with 25 μL of substrate Gly-Pro-*p*-nitroanilide (12 mM) at 37 °C for 10 min, after which 50 μL of DPP-IV (0.02 unit/mL) was added and the mixture incubated at 37 °C for 30 min. The enzymatic reaction was terminated by the addition of 100 μL of 1 M sodium acetate buffer, pH 4.0, and the absorbance of the released *p*-nitroanilide was measured at 405 nm using a Labsystems iEMS Reader MF (Labsystems Oy, Helsinki, Finland). Each test sample was analyzed in triplicate, and the absorbance values were corrected for sample blanks in which DPP-IV was replaced with Tris-HCl buffer (100 mM, pH 8.0). The positive control (DPP-IV activity with no inhibitor) and negative control (no DPP-IV activity) were prepared by using Tris-HCl buffer (100 mM, pH 8.0) in place of the sample and in place of the sample and DPP-IV solution, respectively. The percent DPP-IV inhibition was calculated as follows:

$$\%\ \text{DPP-IV inhibition} = 100 \times \{1 - [(A_{405}(\text{test sample}) - A_{405}(\text{test sample blank})]/[(A_{405}(\text{positive control}) - A_{405}(\text{negative control})]\}$$

The IC_{50} values (concentrations of hydrolysate required to cause a 50% inhibition of the enzyme activity) were determined from the cubic regression equations generated by fitting the data from the plot of percent DPP-IV inhibition against hydrolysate concentrations ranging from 1.25 to 0.0125 mg/mL (final assay concentration). The tripeptide diprotin A (Ile-Pro-Ile) was used as a reference inhibitor.

Extraction of Rat Intestinal α-Glucosidase. α-Glucosidase was extracted from rat intestine acetone powder as described by Liu et al.[37] Briefly, 0.5 g of rat intestine acetone powder was suspended in 20 mL of 0.1 M sodium phosphate buffer, pH 6.9, and stirred for 30 min at 4

°C. The mixture was then centrifuged at 2000*g* at 4 °C for 10 min, and the supernatant was collected and kept at −18 °C for further use.

Determination of α-Glucosidase Inhibitory Activity. The inhibitory activity of the hydrolyzed whey protein samples was assessed by comparing the amount of glucose released by the α-glucosidase enzyme from its natural substrate, maltose, in the presence and absence of hydrolyzed samples.[37]

The hydrolyzed whey protein samples were diluted in 0.1 M sodium phosphate buffer, pH 6.9, to the desired concentrations. The sample solutions (150 μL) were preincubated with 150 μL of rat α-glucosidase solution (obtained as described above and diluted 40 times with 0.1 M sodium phosphate buffer, pH 6.9) for 5 min at 37 °C in a shaker incubator (New Brunswick Scientific; Enfield, CT, USA) prior to the addition of 300 μL of 20 mg/mL maltose solution (in ddH_2O). The mixtures were incubated at 37 °C with agitation for 6 min and then submerged in boiling water for 4 min to stop the enzymatic reaction. Aliquots of the inactivated suspensions (100 μL) were incubated with 200 μL of glucose assay reagent (prepared according to the supplier's instructions) at 37 °C for 30 min. The reaction was then stopped by the addition of 200 μL of 6 M sulfuric acid, and the mixtures were centrifuged for 5 min at 14000 rpm. The supernatants (200 μL) were pipetted into a 96-well microplate, and the absorbance of the samples was measured at 540 nm. The positive control (α-glucosidase activity with no inhibitor) was prepared by using 150 μL of 0.1 M sodium phosphate buffer, pH 6.9, in place of the sample solution. Each test sample was analyzed in triplicate. The concentrations of glucose produced from the substrate by the α-glucosidase enzyme were determined from a glucose calibration curve prepared using the glucose assay kit. The percent α-glucosidase inhibition was calculated as

$$\%\ \alpha\text{-glucosidase inhibition} = 100 \times \{1 - ([\text{glucose}]_S/[\text{glucose}]_B)\}$$

where $[\text{glucose}]_S$ and $[\text{glucose}]_B$ represent the concentration of glucose produced in the presence and absence of test sample, respectively.

The IC_{50} values were determined from the cubic regression equations obtained by fitting the data from the plot of the percent α-glucosidase inhibition against hydrolysate concentrations ranging from 2.5 to 7.5 mg/mL (final assay concentration). Acarbose was used as a reference inhibitor.

Statistical Analysis. To compare the extents of hydrolysis and DPP-IV or α-glucosidase inhibitory activities of the hydrolyzed whey protein samples, one-way analysis of variance using the general linear model and pairwise comparison with Tukey's method were performed using Minitab statistical software (version 16, Minitab Inc., State College, PA, USA). All experiments were conducted at least in triplicate. Data are expressed as the mean ± standard deviation (SD), and significant difference was established at $P < 0.05$.

RESULTS AND DISCUSSION

Extent of Hydrolysis of Pepsin-Treated Whey Proteins. The extent to which the whey proteins were hydrolyzed by pepsin during the enzymatic hydrolysis treatment was evaluated. As shown in Figure 1, the extent of hydrolysis differed significantly between the pepsin-treated whey proteins. The complete hydrolysis of whey proteins to their constituent amino acids has been reported to yield 8.8 mmol of amino acids/g of protein.[38] With the extent of hydrolysis values all lower than 1.0 mmol L-leucine equiv/g of protein, none of the whey proteins were completely hydrolyzed by the peptic treatment used in this study. Among the four individual proteins investigated, the serum albumin hydrolysate displayed the highest content of α-amino groups, whereas the lowest content was observed for the hydrolysate of β-lactoglobulin. Moreover, SDS-PAGE of the pepsin-treated β-lactoglobulin (Figure 2B, lane 3) revealed that it contained intact proteins and fewer low molecular weight bands than the pepsin-treated

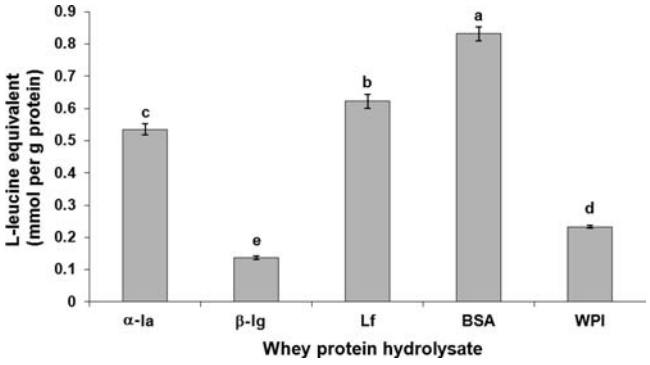

Figure 1. Extent of hydrolysis (mmol L-leucine equiv/g protein) of whey protein hydrolysates obtained by peptic treatment. Each bar represents the mean and standard deviation of three determinations. Bars labeled with different letters are significantly different ($P < 0.05$). α-la, α-lactalbumin; β-lg, β-lactoglobulin; Lf, lactoferrin; BSA, bovine serum albumin; WPI, whey protein isolate.

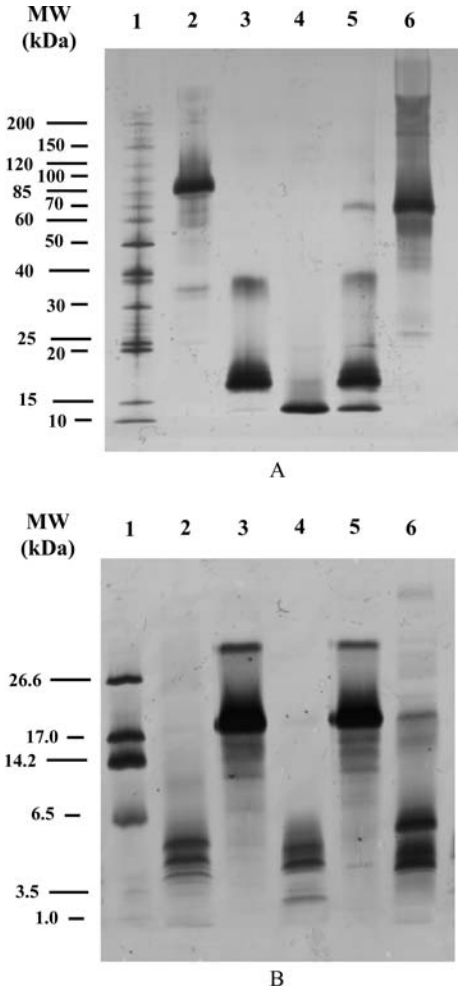

Figure 2. SDS-PAGE electrophoregrams of whey protein samples (A) and their hydrolysates after peptic treatment (B). Lanes: 1, molecular weight markers; 2, lactoferrin; 3, β-lactoglobulin; 4, α-lactalbumin; 5, whey protein isolate; 6, bovine serum albumin.

lactoferrin, α-lactalbumin, and serum albumin samples (Figure 2B, lanes 2, 4, and 6, respectively). The resistance of native β-lactoglobulin to pepsin digestion has been previously reported and suggested to be caused by the folded calyx structure of this

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protein, making target peptide bonds unavailable to proteolytic action.[39] Although mainly composed of β-lactoglobulin (Figure 2A), WPI had an extent of hydrolysis almost 2 times higher (Figure 1). It is possible that the process used to produce the WPI may have caused changes to the proteins' structure and consequently affected their susceptibility to proteolytic cleavage.

DPP-IV Inhibitory Activity of Pepsin-Treated Whey Proteins. The DPP-IV inhibitory activity of the whey protein hydrolysates obtained by peptic treatment was investigated. As shown in Figure 3, when present at a concentration of 0.500 mg/mL, all whey protein hydrolysates obtained after 60 min of digestion with pepsin were able to cause the inhibition of the DPP-IV enzyme. The α-lactalbumin and WPI hydrolysates, with 91 and 82% inhibition, respectively, showed the highest DPP-IV inhibitory activity, whereas the pepsin-treated β-lactoglobulin sample was the least effective at inhibiting the activity of the enzyme (28% inhibition). Unhydrolyzed proteins, on the other hand, displayed no inhibitory activity (data not shown). The IC_{50} value measured for the α-lactalbumin hydrolysate (0.036 mg/mL) was found to be of the same order of magnitude as the IC_{50} of WPI hydrolysate, but about 10 times lower than that observed for the serum albumin and lactoferrin hydrolysates and roughly 35 times lower than that for the β-lactoglobulin hydrolysate (Table 1). The relatively low inhibitory activity of the hydrolyzed β-lactoglobulin sample compared to the WPI hydrolysate is surprising because β-lactoglobulin is the major protein fraction in whey (Figure 2). To assess whether the low DPP-IV inhibitory activity of the pepsin-treated β-lactoglobulin could be the result of the resistance of this protein to being hydrolyzed, a 3% β-lactoglobulin solution was heated at 90 °C for 15 min prior to the hydrolysis treatment with pepsin. Heating β-lactoglobulin at 80−90 °C has been shown to cause structural changes that improve the susceptibility of its peptide bonds to peptic digestion.[39] The heating pretreatment did increase the extent of hydrolysis of the β-lactoglobulin hydrolysates to 0.75 mmol L-leucine equiv/g protein, but had no significant effect on the DPP-IV inhibitory activity (data not shown). The IC_{50} value for the pepsin-treated β-lactoglobulin found in this study is of the same order as the value reported by Uchida et al.[21] for a β-lactoglobulin hydrolysate obtained by enzymatic hydrolysis with the digestive enzyme trypsin (IC_{50} = 210 μM or ~3.9 mg/mL). The authors isolated and identified the hexapeptide Val-Ala-Gly-Thr-Trp-Tyr (IC_{50} = 174 μM or ~0.12 mg/mL) as being the active component responsible for the inhibitory activity of the tryptic hydrolysate.[21]

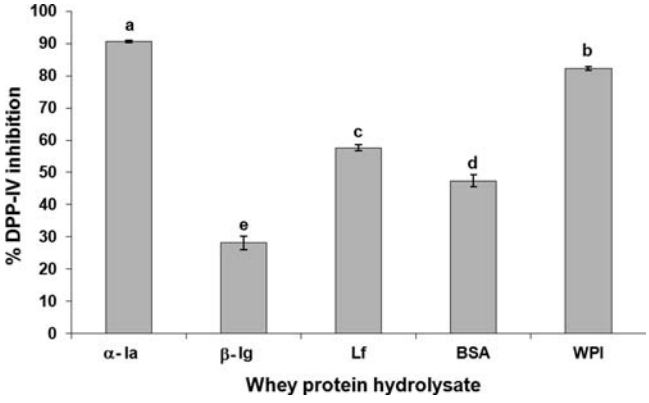

Figure 3. DPP-IV inhibitory activity of whey protein hydrolysates obtained by peptic treatment. The percent inhibition of DPP-IV was measured using 0.500 mg/mL of hydrolysate (final assay concentration, protein basis). Each bar represents the mean and standard deviation of three determinations. Bars labeled with different letters are significantly different ($P < 0.05$). α-la, α-lactalbumin; β-lg, β-lactoglobulin; Lf, lactoferrin; BSA, bovine serum albumin; WPI, whey protein isolate.

Table 1. DPP-IV and α-Glucosidase Inhibitory Activities of Hydrolyzed Whey Proteins Obtained by Peptic Treatment

	IC_{50}[a] (mg/mL)	
hydrolysate	DPP-IV	α-glucosidase
α-lactalbumin	0.036 ± 0.002 c	ND
β-lactoglobulin	1.279 ± 0.100 a	3.5 ± 0.4 a
lactoferrin	0.379 ± 0.035 b	ND
bovine serum albumin	0.513 ± 0.056 b	ND
whey protein isolate	0.075 ± 0.006 c	4.5 ± 0.6 a
diprotin A[b]	0.0016 ± 0.0003	NA
acarbose[b]	NA	$2.8 \times 10^{-4} \pm 0.3 \times 10^{-4}$

[a]IC_{50} values are reported as the mean and standard deviations from triplicate determinations and expressed at final assay concentration. Within the same column, figures with different lower case letters are significantly different ($P < 0.05$). NA, not applicable; ND, not determined. [b]Diprotin A and acarbose were used as reference inhibitors for DPP-IV and α-glucosidase, respectively.

Peptides with different biological properties, including opioid and ACE inhibitory activities, have been found in α-lactalbumin hydrolysates obtained by peptic digestion.[40] However, to our knowledge, the generation of peptides with DPP-IV inhibitory activity from α-lactalbumin has never been reported. α-Lactalbumin is the second main constituent of WPI, accounting for about 14% of its total protein content (Figure 2A). The similar inhibitory activities observed for α-lactalbumin and WPI hydrolysates suggest that α-lactalbumin-derived peptides might be among the active components responsible for the inhibition of DPP-IV activity observed with the WPI hydrolysate.

The hydrolysates obtained by the peptic digestion of lactoferrin and serum albumin showed similar DPP-IV inhibitory activities (Table 1). A lactoferrin hydrolysate of comparable potency (IC_{50} = 1.088 mg/mL), obtained using a food grade commercial gastrointestinal preparation, was also recently reported in a study by Nongonierma and FitzGerald.[24]

α-Glucosidase Inhibitory Activity of Pepsin-Treated Whey Proteins. The whey protein hydrolysates obtained by peptic digestion were also assessed for their inhibitory activity against the α-glucosidase enzyme. Most in vitro studies on the α-glucosidase inhibitory activity of natural compounds have used microbial α-glucosidase (from baker's yeast).[33] However, the magnitude of α-glucosidase inhibition has been reported to be greatly affected by the origin of the enzyme. The synthetic α-glucosidase inhibitors voglibose and acarbose were shown in a study by Oki et al.[41] to strongly inhibit the activity of mammalian α-glucosidase, but to have little or no effect on baker's yeast α-glucosidase. On the other hand, (+)-catechin as well as some food products, such as yogurt, chicken essence, and fish sauce, showed inhibitory activity against yeast α-glucosidase but not against the mammalian enzyme.[41] It has been suggested that altered binding of the inhibitors to the active site, which may arise from structural differences among the enzymes, could be responsible for these conflicting findings.[41,42] For the investigation of the α-glucosidase

inhibitory activity of the whey protein hydrolysates to have better relevance to human health, mammalian (rat) intestinal α-glucosidase was used in the present study. Moreover, maltose was chosen as the substrate for the enzyme assay in this study because it is the major digestive product of starch in the small intestine.[30]

The inhibition of α-glucosidase activity in the presence of whey protein hydrolysates at 2.50 mg/mL was investigated. Of the five samples, β-lactoglobulin and WPI hydrolysates displayed the highest α-glucosidase inhibitory activity, with 33 and 36% inhibition, respectively (Figure 4). The α-lactalbumin

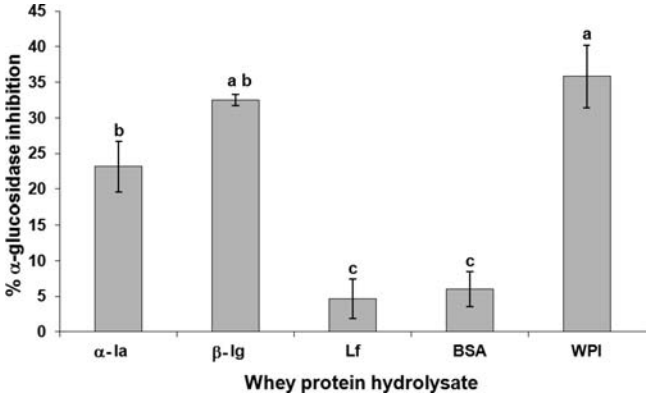

Figure 4. α-Glucosidase inhibitory activity of whey protein hydrolysates obtained by peptic treatment. The percent inhibition of α-glucosidase was measured using 2.50 mg/mL of hydrolysate (final assay concentration, protein basis). Each bar represents the mean and standard deviation of three determinations. Bars labeled with different letters are significantly different ($P < 0.05$). α-la, α-lactalbumin; β-lg, β-lactoglobulin; Lf, lactoferrin; BSA, bovine serum albumin; WPI, whey protein isolate.

hydrolysate was also able to inhibit the activity of α-glucosidase but not as effectively (24% inhibition), whereas the hydrolyzed lactoferrin and serum albumin samples had low inhibitory activity (5 and 6% inhibition, respectively) (Figure 4). None of the unhydrolyzed proteins were able to inhibit the α-glucosidase enzyme (data not shown). The IC_{50} values of the hydrolyzed β-lactoglobulin and WPI samples (3.5 and 4.5 mg/mL, respectively) were not found to be significantly different (Table 1). Similarly to the results for DPP-IV inhibitory activity of the hydrolysates relative to the reference inhibitor diprotin A, the α-glucosidase inhibitory activity of the whey protein hydrolysates was significantly lower than that measured for the reference inhibitor acarbose (IC_{50} = 0.28 μg/mL). Because the protein hydrolysates are composed of a mixture of peptides, only some of which would display inhibitory activity, their lower potency compared to isolated peptides or synthetic drugs is not surprising.

Although the majority of synthetic α-glucosidase inhibitors, such as acarbose and voglibose, are sugars or derivatives of sugar moieties,[43] most α-glucosidase inhibitory compounds reported to be derived from natural sources are of phenolic nature, including flavonoids and tannins.[32,44,45] On the other hand, a few peptides isolated from food proteins of animal origin have also been reported to inhibit the activity of α-glucosidase.[33,34,46] In a survey of the in vitro α-glucosidase inhibitory activity of different food commodities, Matsui et al.[33] showed that an alkaline protease hydrolysate from sardine muscle was able to inhibit the carbohydrate-hydrolyzing enzyme with an IC_{50} value of 48.7 mg/mL. Upon further fractionation of the sardine muscle hydrolysate, the authors isolated and identified the α-glucosidase inhibitory peptides Val-Trp (IC_{50} = 22.6 mM or ∼6.9 mg/mL) and Tyr-Tyr-Pro-Leu (IC_{50} = 3.7 mM or ∼2.1 mg/mL).[46] More recently, the hexapeptide Arg-Val-Pro-Ser-Leu-Met (IC_{50} = 23.07 μM or ∼0.016 mg/mL) and the pentapeptide Thr-Pro-Ser-Pro-Arg (IC_{50} = 40.02 μM or ∼0.022 mg/mL), derived from egg white protein, were reported to possess α-glucosidase inhibitory activity.[34] To the best of our knowledge, the present study is the first to report the generation of α-glucosidase inhibitory activity by proteolysis of whey proteins. The exact mechanism by which peptides could inhibit α-glucosidase activity is unknown, but it has been suggested that nonsaccharide compounds may exert their inhibitory activity by binding to the enzyme's active site via hydrophobic interactions.[43]

In summary, results from the present study demonstrated that peptides with inhibitory properties against DPP-IV and α-glucosidase activities can be generated from the peptic digestion of whey proteins. Whereas WPI, α-lactalbumin, and β-lactoglobulin hydrolysates displayed both DPP-IV and α-glucosidase inhibitory activities, products obtained by hydrolysis of lactoferrin and serum albumin were able to inhibit only the DPP-IV enzyme. These findings suggest that whey protein hydrolysates with DPP-IV and α-glucosidase inhibitory activities may have the potential to improve blood glucose regulation by means of their ability to slow both the inactivation of the incretin hormones and the intestinal digestion of carbohydrates. Additional investigations to isolate and sequence the bioactive peptides present within dairy protein hydrolysates would allow a better understanding of their modes of action on DPP-IV and α-glucosidase activities. Furthermore, clinical studies are also needed to confirm the efficacy of these whey protein-derived peptides in humans.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

α-la, α-lactalbumin; β-lg, β-lactoglobulin; BSA, bovine serum albumin; DPP-IV, dipeptidyl peptidase-IV; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IC_{50}, concentration causing 50% inhibition; Lf, lactoferrin; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; WPI, whey protein isolate

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