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Modification of Vegetable Proteins to Release Bioactive Peptides Able to Treat Metabolic Syndrome—In Silico Assessment

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Featured Application: This work introduces a novel way of designing proteins for the treatment of diseases, especially metabolic syndrome.

Abstract: Metabolic syndrome comprises a cluster of diseases like hypertension, dyslipidemia, and insulin resistance, among others. Its treatment is based on lifestyle modification; however, this treatment often fails to improve metabolic syndrome indicators over the long term. In this work, sequences of some representative vegetable proteins were explored to find bioactive peptides with activity toward metabolic disorders of metabolic syndrome. Five proteins, i.e., legumin (chickpea), glutelin type A-2 (chickpea), glutelin type B-2 (rice), prolamin PPROL 17 (maize), and glutelin (rice) revealed a high potential to be effective against metabolic syndrome. We designed and evaluated in silico modifications to their amino acid sequence to release bioactive peptides after simulating gastrointestinal digestion (SGD). The approach presented here allows the design of proteins that could combat metabolic syndrome, for later production and study. In the future, these proteins can be used as functional foods.

Keywords: metabolic syndrome; bioactive peptides; storage proteins; in silico design

1. Introduction

Metabolic syndrome is a cluster of metabolic disturbances that increase the risk of stroke and cardiovascular diseases (CVD). Obesity, hypertension, insulin resistance, and dyslipidemias are the principal components of metabolic syndrome [1]. This syndrome affects pediatric populations [2] as well as older people [3] and is a public health issue. The current treatment is based on lifestyle interventions, particularly by modification of the diet and an increase in physical activity to prevent cardiovascular complications [4]. Drugs can also be used to treat unique components of the syndrome [5]. The principal aim of treatment is to prevent or delay the complications of CVD, but this primary intervention is only effective for weight control [6].

An alternative metabolic syndrome treatment is the use of bioactive peptides—short sequences of amino acids that improve human health. These peptides can be released from both animal and vegetable proteins, and more than 1500 sequences have been reported [7]. Bioactive peptides have shown multiple beneficial effects on human health such as antihypertensive, antidiabetic, antimicrobial, immunomodulatory, anti-cancer, lipid-lowering, antioxidant effects, and dipeptidyl-peptidase 4

(DPPIV) inhibition. Some of these are useful in the treatment of metabolic syndrome [8,9], so bioactive peptides or proteins that contain these peptides can be part of functional foods [10].

In silico analysis is a valuable tool for predicting specific functions and/or for establishing strategies to deliver bioactive peptides from proteins. For example, Lin et al. [11] analyzed αs_1, αs_2, β, and κ-casein from yak milk and found that these proteins have peptidic fragments in their sequence with reported biological activity, like angiotensin-converting-enzyme inhibitors (ACEI) and DPPIV inhibitors. They proposed a strategy to release them with a tandem digestion; similarly, Fu et al. [12] evaluated the potential of releasing bioactive peptides from patatin after in silico digestion with different proteases and they found multiple fragments with ACEI and DPPIV activity.

Bioactive peptides can be obtained by chemical synthesis; nevertheless, the production process can be non-viable [13]. Two principal methods are used to produce bioactive peptides. In the first, a protein or protein mixture is subjected to hydrolysis with one or more proteolytic enzymes. The other production method is microbial fermentation and involves the use of bacteria or yeast, rather than pure or crude enzymes, to hydrolyze proteins in the culture broth [8]. In both cases, the starting material to be hydrolyzed consists of food proteins. Clearly, the amount and type of bioactive peptides than can be obtained by such methods are determined by the amino acid sequences of the starting material. Here, we propose the use of protein engineering methods to increase the number and diversity of bioactive peptides that can be obtained via the hydrolysis of a particular protein. To do this, we first defined a set of bioactive peptides that can treat metabolic syndrome. We then evaluated a group of representative vegetable proteins to determine the number and type of bioactive peptides included in their natural sequences. Considering that plants provide approximately 58% of proteins that humans consume [14], seeds are the main source of protein consumed by humans [15]. Interestingly, proteins from plants are associated with a lower incidence of CVD [16]. Thus, seed storage proteins were appraised in this work. We later designed mutations to increase the number and type of bioactive peptides. Finally, we evaluated the digestion patterns of the modified proteins in silico. These proteins can be produced by heterologous expression in bacteria or yeast.

2. Materials and Methods

2.1. Bioactive Peptides Selection

ACEI peptides and inhibitors of DPPIV peptides were selected from the BIOPEP database [17]; only di- and tripeptides were selected. These peptides were sorted on the basis of their reported IC_{50}, and those peptides with in vivo activity were given priority. Antioxidant and lipid-lowering peptides were searched and selected based on the literature. To evaluate resistance to gastrointestinal enzymes, the selected peptides were submitted to in silico gastrointestinal digestion with BIOPEP and PeptideCutter tools, choosing pepsin pH 1.3, trypsin, and chymotrypsin.

2.2. Scaffold Selection

The sequences of some representative seed storage proteins were obtained from the UniProt Database to identify the previously selected peptides; protein sequences were scanned, and their potential to treat metabolic syndrome was evaluated with the parameter A, defined as:

$$A = a/N$$

Here, A is the relative frequency of bioactive peptides able to treat metabolic syndrome, a is the number of selected bioactive peptides, and N is the sequence length.

2.3. Protein Design

To design proteins capable of treating metabolic syndrome, we considered the specificities of gastrointestinal enzymes such as pepsin, trypsin, and chymotrypsin. We then proposed modifications

to their amino acid sequences to release the selected peptides after simulating gastrointestinal digestion. The in silico peptide profiles of modified proteins were then compared with those of the native ones after digestion with the aforementioned enzymes. To design the proteins, sequences were used without the signal peptide.

2.4. In Silico Evaluation

The modified proteins were evaluated by the Peptide Cutter tool to determine the release of the biopeptides; protein toxicity was evaluated using the ToxinPred tool (Bioinformatics Centre, CSIR-Institute of Microbial Technology, Chandigarh, India, 2013) available in http://crdd.osdd.net/raghava/toxinpred/ with default options [18].

3. Results and Discussion

3.1. Peptides to Treat Metabolic Syndrome

Proteins from foods are digested in the gastrointestinal tract by multiple proteases. Total protein is digested to di- and tripeptides before being absorbed by small intestine cells. Only 10% of the protein remains as di- and tripeptides after digestion by peptidases of the brush border and enterocytes [19]. Thus, we only considered peptides with a maximum length of three amino acids to construct a protein that can release these peptides. The BIOPEP database showed 258 ACEI peptides and 287 DPPIV inhibitor peptides. These peptides were sorted according to their IC_{50} and evaluated in gastrointestinal digestion (SGD). ACEI peptides that showed resistance to SGD and presented the highest activity were selected. These were IW (isoleucine-tryptophan), VY (valine-tyrosine), IY isoleucine-tyrosine, EY (glutamate-tyrosine), and DG (aspartate-glycine). Except for EY, all peptides presented in vivo activity despite being hydrophobic [20–23]. These have been described as unstable during gastrointestinal digestion [24]. For protein design, we selected the tripeptide IPI (isoleucine-proline-proline) as a DPPIV inhibitor because it is the most powerful peptide against this enzyme [25]. Based on the review by Nogonierma [26], the antioxidant dipeptide CG [27] and the lipid-lowering dipeptide DE [28] were also chosen as potential peptides against metabolic syndrome (Table 1). Iwaniak [9] also recently reviewed peptides with the potential to treat metabolic syndrome, including the aforementioned peptides. This work suggests that the dipeptides IY and VY are potential peptides to treat hypertension in metabolic syndrome. Most of the selected peptides considered for protein design are uncharged or hydrophobic. This could favor their bioavailability and absorption through the transporter PepT1 and paracellular transport [29].

Table 1. Biopeptides selected to design a protein for metabolic syndrome treatment.

Peptide	Activity	IC50 (μM)	In Vivo	Reference
VY	ACEI	7.1	✓	[30]
VW	ACEI	1.4	x	[31]
IW	ACEI	4.7	✓	[20]
IY	ACEI	2.69	✓	[32]
EY	ACEI	2.68	x	[33]
DG	ACEI	12.3	✓	[22]
IPI	DPPIV Inhibitor	1.1 μg/ml	x	[26]
GW	Antioxidant	-	x	[34]
DW	Antioxidant	-	x	[35]
CG	Antioxidant	-	x	[24]
DE	Lipid-lowering	-	✓	[28]

In vivo: ✓ Indicates peptides with a reported in vivo activity; x indicates no reported in vivo activity. ACEI, angiotensin-converting-enzyme inhibitors, DPPIV, dipeptidyl-peptidase 4.

3.2. Scaffold Selection

We obtained 41 sequences of seed storage proteins from different vegetables from the Uniprot Database. To evaluate their therapeutic potential, the amino acid sequences of the selected bioactive peptides were searched (Table 2). The protein with the highest number of selected peptides (11) was rice glutelin (Q6T725). Higher *A* values were found for rice glutelin Q6T725 (0.023), chickpea legumin (Q9SMJ4) (0.021), chickpea glutelin A-2 (A0A1S2YJV5) (0.03), maize prolamin PPROL 17 (B6UH22) (0.024), and rice glutelin B-2 (Q02897) (0.021).

The highest *A* value was found in chickpea glutelin A-2 and maize prolamin PPROL 17; however, these proteins had the lowest number of selected peptides, six and four, respectively. Their high *A* value is a consequence of a lower number of amino acids. The five proteins selected as scaffolds contain peptides with ACEI activity—all of them present the dipeptide VY, except chickpea legumin. This VY dipeptide is the most studied antihypertensive peptide and has in vivo activity [22]. Maize prolamin PPROL 17 is the only protein containing the antioxidant peptide CG. The DE lipid-lowering peptide was found in three of the selected proteins, in particular, in chickpea legumin, where it was found seven times. Thus, this protein can offer lipid-lowering activity after modifications. None of the selected proteins contain the DPPIV inhibitor peptide IPI.

3.3. In Silico Design of a Protein against Metabolc Syndrome

Five of the selected proteins were then analyzed by the SGD with the PeptideCutter too, l as described in the Materials and Methods section. Despite the fact that each protein has multiple hydrolysis sites for the enzymes employed, only four of the selected peptides were released: one from rice glutelin, one from chickpea glutelin A-2, and two from rice glutelin B-2. In all cases, the released peptide was the ACEI peptide VY. To facilitate the release of the peptides of interest, we leveraged the specificities of digestive enzymes and designed linker sites substituting amino acids with Phe, which is hydrolyzed at the C-terminus, or Leu, which is hydrolyzed at both the C-terminus and the N-terminus (Figure 1). These substitutions affected residues adjacent to the peptides of interest. Arg and Lys were used as linkers in the N-terminus to substitute hydrophilic or charged amino acids.

Chickpea legumin has two DEDEDE hexapeptides in positions 251–256 and 267–272. Here, the proposed modifications were the addition of Leu between each DE repeat. As expected, each of the peptides were released subsequent to SGD after the proposed modifications (Table 3). The rice glutelin residues 244–246 are DEY, and the dipeptides DE and EY are both present in this sequence. We designed a site to release the DE dipeptide because of the high number of ACEI peptides found in this protein.

Pooj [35] reported that glutelin type A-1 releases multiple ACEI peptides after pepsin digestion; this protein has 95% identity with glutelin type A-2 analyzed here. For none of the other proteins investigated, biological activity predictions derived from bioactive peptides have been published. To the best of our knowledge, this is the first work that proposes modification of proteins to release bioactive peptides after oral ingestion. This can enhance and/or provide specific biological activity to proteins in addition to their nutritional value. Toxicity analyses did not show sequences or motifs indicating toxicity after the proposed substitutions.

This work reports a new approach for the use of bioactive peptides as an alternative treatment for metabolic syndrome. Further experimental confirmation is required, because proteolytic enzymes do not always behave as expected. This type of analysis speeds up the design of therapeutic proteins or released peptides. To the best of our knowledge, there are no reported cases of proteins designed with the ability to treat or prevent metabolic syndrome.

Table 2. Frequency of the selected bioactive peptides in vegetable proteins and their *A* number (relative frequency of bioactive peptides able to treat metabolic syndrome).

	Uniprot Entry	Sequence Lenght	IW	VW	VY	IY	EY	DG	IPI	GW	CG	DW	DE	Total	*A*
Soy															
β-conglicinin chain alfa	P11827	617	0	0	0	1	0	0	0	0	0	0	6	7	0.011
β-conglicinin chain beta	P25974	426	0	0	0	1	0	0	0	0	0	0	2	3	0.007
Albumin 2S	P19594	137	0	0	0	0	0	0	0	0	0	0	2	2	0.015
Basic 7S Globulin	P13917	403	0	1	1	0	1	1	0	0	1	0	0	5	0.012
Amaranth															
11S Globulin	Q38712	501	0	1	1	2	1	1	0	0	0	0	2	8	0.016
Oat															
Avenin 3	P80356	201	0	0	1	0	0	0	0	0	0	0	0	1	0.005
11S Globulin	Q38780	503	0	0	2	2	1	1	0	0	0	0	2	8	0.016
Beans															
Phaseolin	P80463	404	0	0	2	1	0	0	0	0	0	0	0	3	0.007
Globulin-1	A6YNT0	224	0	0	0	0	0	1	0	0	0	0	2	3	0.013
Alpha-zein 16	P04700	242	0	0	1	0	0	0	0	0	0	0	0	1	0.004
Gamma-zein	C0P381	267	0	0	0	1	0	0	0	0	2	0	0	3	0.011
Rice															
Prolamin PPROLINE 4E	Q0DJ45	131	0	1	0	1	0	0	0	0	0	0	0	2	0.015
Cupincin	B8AL97	436	0	0	1	0	1	0	0	0	0	0	5	7	0.016
Globulin	P29835	164	0	0	0	0	1	0	0	1	0	0	0	2	0.012
Glutelin	Q6T725	471	0	0	3	1	2	2	0	0	0	0	3	11	0.023
Glutelin Type A-2	P07730	475	0	0	3	1	0	1	0	0	0	0	3	8	0.017
Glutelin Type B-2	Q02897	481	0	0	4	1	1	2	0	0	0	0	2	10	0.021
Maize															
Globulin-1 S Allele	P15590	487	0	0	0	0	0	1	0	0	0	0	2	3	0.006
22 kDa alpha zein 4	O48966	245	0	0	0	0	0	0	0	0	0	0	0	0	0.000
50 kDa gamma zein	C0P381	267	0	0	0	1	0	0	0	0	2	0	0	3	0.011
Globulin-2	Q7M1Z8	431	0	0	1	0	0	0	0	0	0	0	3	4	0.009
Globulin-1	A6YNT0	224	0	0	0	0	0	1	0	0	0	0	2	3	0.013
18 kD delta zein	Q946V9	190	0	0	0	0	0	0	0	0	0	0	0	0	0.000
Prolamin PPROL 17	B6UH22	164	0	0	1	0	0	0	0	0	3	0	0	4	0.024
Chickpea															
Legumin	Q9SMJ4	475	1	0	0	1	0	1	0	0	0	0	7	10	0.021
Globulin-1 S Allele	A0A1S2YZ56	621	0	0	0	1	1	2	0	1	1	0	3	9	0.014
11S Globulin seed storage	A0A1S2YGT3	348	1	1	2	0	0	2	0	0	0	0	1	7	0.020

Table 2. *Cont.*

	Uniprot Entry	Sequence Lenght	IW	VW	VY	IY	EY	DG	IPI	GW	CG	DW	DE	Total	*A*
Glutelin Type-A 2-Like	A0A1S2YJV5	200	0	1	1	0	0	4	0	0	0	0	0	6	0.030
Lentil															
Albumin S	P86782	37	0	0	0	0	0	0	0	0	0	0	0	0	0.000
Broad Bean															
Legumin type B	P05190	462	0	0	0	2	2	1	0	0	0	0	1	6	0.013
Vicilin	P08438	436	0	0	0	1	2	0	0	0	0	0	2	5	0.011
Convicilin	B0BCL8	469	0	0	0	0	1	0	0	0	0	0	4	5	0.011
Wheat															
Glutenin subunit DX5	P10388	827	0	0	0	0	0	0	0	0	0	0	0	0	0.000
Avenin-like B1	Q2A783	267	0	0	0	2	0	0	0	0	0	0	0	2	0.007
Alpha/Beta Gliadin	P02863	266	0	0	1	0	0	0	0	0	0	0	0	1	0.004
Alpha/Beta Gliadin A-I	P04721	242	0	0	0	0	0	0	0	0	0	0	0	0	0.000
Alpha/Beta Gliadin A-II	P04722	271	0	0	1	0	0	0	0	0	0	0	0	1	0.004
Alpha/Beta Gliadin A-III	P04723	262	0	0	1	0	0	0	0	0	0	0	0	1	0.004
Alpha/Beta Gliadin A-IV	P04724	277	0	0	1	0	0	0	0	0	0	0	0	1	0.004
Alpha/Beta Gliadin A-V	P04725	299	0	0	1	0	0	0	0	0	0	0	0	1	0.003
Glutelin Type A-1	M7ZVJ6	299	0	1	0	0	0	3	0	0	1	1	0	6	0.020

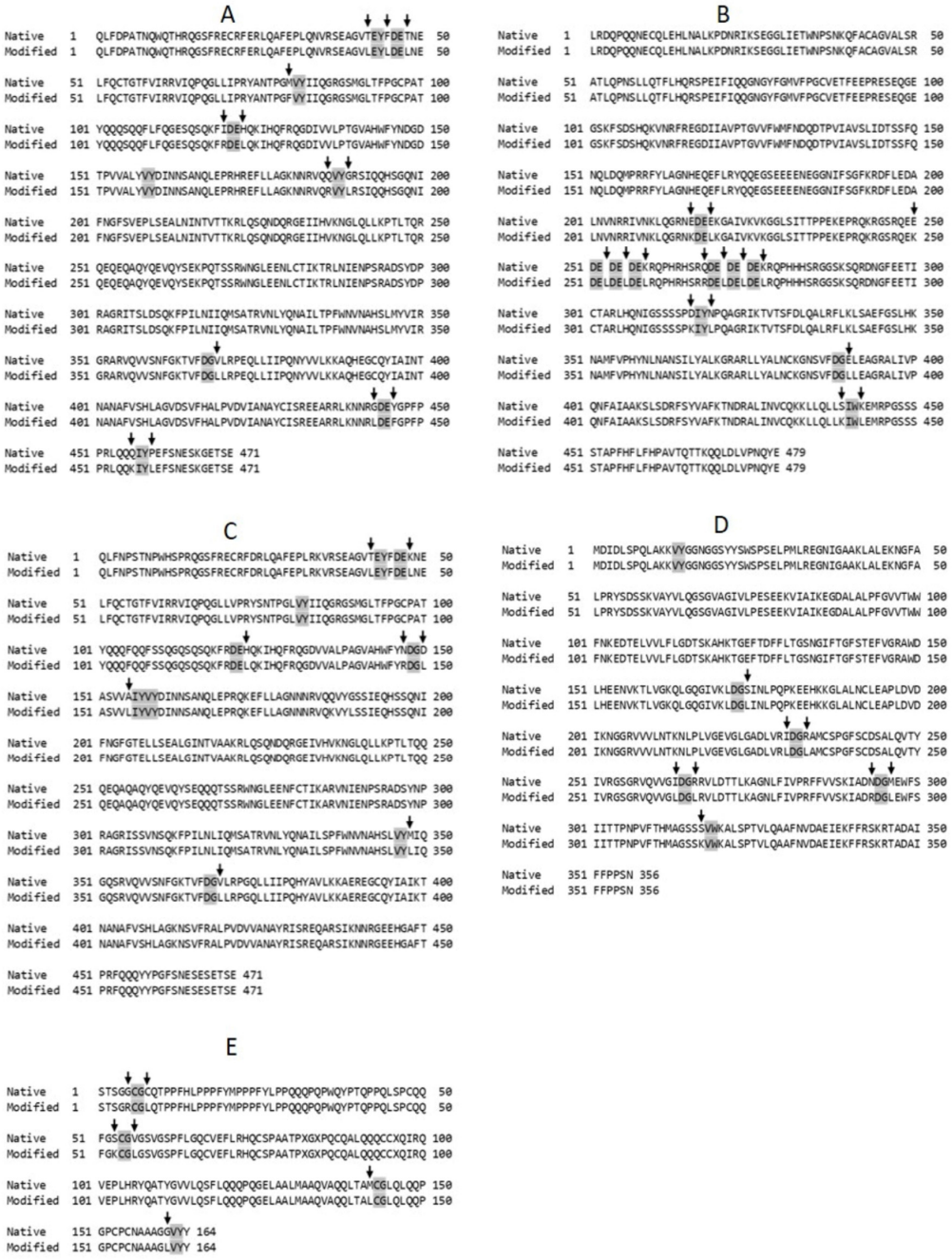

Figure 1. Sequences of (**A**) rice glutelin Q6T725, (**B**) chickpea legumin Q9SMJ4, (**C**) chickpea glutelin type B-2 Q02897, (**D**) chickpea gutelin type A-2 A0A1S2YJV5, and (**E**) maize prolamin PPROL 17 B6UH22 and their modifications. The gray squares indicate selected bioactive peptides, and the arrows indicate modifications in the amino acid sequences.

Table 3. Number of peptides released after modifications.

Protein	Number of Cleavage Sites		Fragments		Selected Peptides Released	
	Native	Modified	Native	Modified	Native	Modified
Glutelin Q6T725	160	182	144	160	1	8
Legumin Q9SMJ4	192	206	167	180	0	10
Glutelin type-A 2 A0A1S2YJV5	143	154	123	134	1	6
Prolamin PPROL 17 B6UH22	42	49	39	47	0	4
Glutelin type-B 2 Q02897	165	179	144	159	2	9

4. Conclusions

The dipeptides identified in the natural proteins reported here are not released after a simulated gastrointestinal digestion. This suggests they would not treat metabolic syndrome. After the design proposed here, rice glutelin type B-2 appears as the best option to treat metabolic syndrome. Chickpea legumin, after the modifications proposed here, appears as an option to diminish cholesterol. The approach described here can facilitate the design of proteins that could combat metabolic syndrome. These proteins can be produced by heterologous expression in bacteria or yeast; in the long term, they could be produced in transgenic plants. These recombinant proteins can be added to food matrices.

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