Structural properties of bioactive peptides with α-glucosidase inhibitory activity

Mohammed Auwal Ibrahim[1,2*], Megan J Bester[3], Albert WH Neitz[1] and Anabella RM Gaspar[1]

[a]Department of Biochemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa
[b]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria
[c]Department of Anatomy, Faculty of Health Sciences, University of Pretoria, Pretoria 002, South Africa

***Correspondence to:**Dr. Mohammed Auwal Ibrahim, Department of Biochemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa E mail: mauwalibrahim@gmail.com or maibrahim@abu.edu.ng

ABSTRACT

Bioactive peptides are emerging as promising class of drugs that could serve as α-glucosidase inhibitors for the treatment of type 2 diabetes. This article identifies structural and physicochemical requirements for the design of therapeutically-relevant α-glucosidase inhibitory peptides. So far, a total of 43 fully sequenced α-glucosidase inhibitory peptides have been reported and 13 of them had IC_{50} values several folds lower than acarbose. Analysis of the peptides indicates that the most potent peptides are tri– to hexapeptides with amino acids containing a hydroxyl or basic side chain at the N-terminal. The presence of proline within the chain and alanine or methionine at the C-terminal appears to be relevant for high activity. Hydrophobicity and isoelectric points are less important variables for α-glucosidase inhibition while a net charge of 0 or +1 was predicted for the highly active peptides. *In silico* simulated gastrointestinal digestion revealed that the high and moderately active peptides, including the most potent peptide (STYV), were gastrointestinally unstable, except SQSPA. Molecular docking of SQSPA, STYV and STY (digestion fragment of STYV) with α-glucosidase suggested that their hydrogen bonding interactions and binding energies were comparable with acarbose. The identified criteria will facilitate the design of new peptide-derived α-glucosidase inhibitors.

KEY WORDS: Amino acids; α-Glucosidase; Bioactive Peptides, Physicochemical properties,Type 2 diabetes

1. INTRODUCTION

Recent global projection of the prevalence of diabetes mellitus by the International Diabetes Federation (IDF) suggests that about 415 million people are affected with the disease and the number is estimated to increase to about 642 million by 2040.[1] The disease is categorized into type 1 and type 2 diabetes(T2D), the latter is the most prevalent accounting for > 90% of all the diabetic cases worldwide. T2D is a complex heterogeneous disorder associated with insulin resistance and partial pancreatic β cells dysfunction which leads to decreased glucose uptake by tissues and consequently leading to hyperglycemia.[2] Hyperglycemia is thus considered as the major pathogenic feature of T2D and is implicated in the pathogenesis of all the secondary (micro and macro) complications associated with the disease.[3]Therefore, the management of postprandial hyperglycemia offers a unique therapeutic option for the treatment of T2D and its associated complications.

α-Glucosidase (EC 3.2.1.20) is a membrane-bound enzyme present in the epithelial mucosa of the small intestine that hydrolyses terminal, non-reducing α(1-4) linked α-glucose residues to release free glucose molecules.[4] Thus, an important therapeutic strategy for the control of postprandial hyperglycemia is by inhibiting the enzymes of carbohydrate digestion such as α-glucosidase and α-amylase.[5] α-Amylase hydrolyses starch and other complex carbohydrates to yield oligosaccharides which are further hydrolysed by α-glucosidase to release glucose. Hence, α-glucosidase inhibitors ultimately impede the uptake of glucose from complex dietary carbohydrates into the blood circulation, thereby effectively diminishing postprandial hyperglycemia.[6]However, multiple side effects such as gastrointestinal disturbances, pain and flatulence have greatly limited the use of the currently available α-glucosidase inhibitors.[7] This has compelled the scientific community to search for novel α-glucosidase inhibitors especially from natural (and easily biorenewable) sources with lower side effects such as plant-based phenolics [8,9] and bioactive peptides.[10, 11]

Bioactive peptides are commonly specific fragments of proteins that elicit biological activity and consequently modulate the health status of individuals.[11]In the last decade, many peptides with different biological activities have been described and are currently receiving great scientific attention whilst new ones are being continuously discovered. This is evident by the propensity of databases and repositories containing huge amount of data on bioactive peptide

structure, properties and activities. Consequently, this has led to the utilization of *in silico* methods to serve as indispensable tools in the manipulation of these peptides for better understanding of physicochemical properties, structure-activity relationships and stability. This information can be used to design peptides with increased bioactivity[12]that can be developed as novel therapeutic agents. Some of the reported bioactivities elicited by bioactive peptides include antibacterial [13-15], antifungal[16],anti-inflammatory [17,18], antioxidant and angiotensin converting enzyme (ACE) inhibitory activities.[19] Other activities attributed to bioactive peptides are anticoagulant [20], anticancer [21], dipeptidyl peptidase IV (DPP-IV) [22] and α-glucosidase inhibitory activities [10]. In this context, peptides with α-glucosidase inhibitory activities are especially becoming important and attractive to food and biopharmaceutical industries because of their high affinity and specificity in addition to low immunogenicity and toxicity profiles.[23] In addition, currently available α-glucosidase inhibitors such as acarbose with characteristic sugar mimetic structures are difficult to prepare by chemical synthesis or to isolate from natural products.[24,25]Therefore, peptides are attractive alternatives as therapeutic agents for α-glucosidase inhibition.

Although increasing number of peptides with α-glucosidase inhibitory activity have been identified, the structural and other physicochemical requirements for optimal activity and effective drug design are poorly understood.The available reviews on antidiabetic peptides were majorly focused on DPP-IV inhibitory peptides.[11, 12, 26]The aim of this article is to identify peptides that inhibit α-glucosidase and address knowledge gaps regarding the physicochemical requirements for inhibition, stability in the gastrointestinal tract (GIT) as well as the structural requirements for effective α-glucosidase inhibition.

2.1 Overall findings on the studies of α-glucosidase inhibitory peptides

To date, a total of 43 fully sequenced peptides have been evaluated for α-glucosidase inhibitory activity (Table 1). Of these peptides, 8 had no activity and 3 were cyclic peptides. Thirteen of the peptides had lower IC_{50} values compared to acarbose (201 μM). The most frequently studied peptides are those with 5 and 6 amino acids(9 peptides each) whilst the longest peptide investigated for α-glucosidase inhibition had 18 amino acid residues. The majority of these peptides were food derived peptides (egg proteinsand sardine muscles) and some were derived from microorganisms such as *Aspergillus* species (Table 1). The α-glucosidase inhibitory activity

Table 1. Sequence, physicochemical characteristics and IC_{50} values of peptides evaluated for α-glucosidase inhibitory activity

Peptide number	Peptide sequence	Chain length	Molecular weight	Net charge	Isoelectric point	Hydrophobicity (%)	Source	Assay method	IC_{50} (μM)	Reference
1	#CL	2	234.32	0	5.11	100	*Aspergillus oryzae*	Y-αG + pNPG	51.63^	Kang et al. [37]
2	#YP	2	278.31	0	6.00	50	Synthetic	Y-αG + pNPG	16800^	Matsui et al. [38]
3	#VW	2	303.37	0	6.09	100	Sardine muscle	Y-αG + pNPG	22600^	Matsui et al.[38]
4	#PL	2	228.30	0	6.09	50	Synthetic	Y-αG + pNPG	No activity	Matsui et al.[38]
5	#PFP	3	359.43	0	6.09	33.33	*Aspergillus oryzae*	Y-αG + pNPG	8.62^	Kang et al.[37]
6	#YPY	3	441.49	0	5.96	66.67	Synthetic	Y-αG + pNPG	25800^	Matsui et al.[38]
7	#YPL	3	391.48	0	6.00	66.67	Synthetic	Y-αG + pNPG	3900^	Matsui et al. [38]
8	#YPG	3	335.37	0	6.00	33.33	Synthetic	Y-αG + pNPG	5000^	Matsui et al.[38]
9	#**STYV**	4	468.50	0	6.02	50	*Aspergillus awamori*	Y-αG + pNPG	**0.012^**	Singh and Kaur[39]
10	#YYPL	4	554.66	0	5.96	75	Sardine muscle	Y-αG + pNPG	3700^	Matsui et al.[38]
11	#QPGR	4	456.52	+1	11.18	0	Silkworm pupae	Y-αG + pNPG	65.8	Zhang et al.[28]
12	#NSPR	4	472.51	+1	11.18	0	Silkworm pupae	Y-αG + pNPG	205	Zhang et al.[28]
13	#QPPT	4	441.5	0	6.09	0	Silkworm pupae	Y-αG + pNPG	560	Zhang et al.[28]
14	**SQSPA**	5	488.50	0	5.24	40	Silkworm pupae	Y-αG + pNPG	**20**	Zhang et al.[28]
15	TPSPR	5	556.62	+1	9.41	40	Egg white protein	Y-αG + pNPG	40.02	Yu et al. [20]
16	KLPGF	5	560.69	+1	8.75	60	Albumin	Y-αG + pNPG	59.5 ± 5.7	Yu et al. [10]
17	EVSGL	5	503.55	-1	4.00	40	Albumin	Y-αG + pNPG	>150.0	Yu et al. [10]
18	EAGVD	5	489.48	-2	3.67	40	Albumin	Y-αG + pNPG	>150.0	Yu et al. [10]
19	HAEIN	5	582.61	-1	5.24	40	Egg white protein	Y-αG + pNPG	>150.0	Yu et al. [20]
20	QIGLF	5	576.69	0	5.52	60	Egg white protein	Y-αG + pNPG	>150.0	Yu et al. [20]
21	DLQGK	5	559.62	0	5.84	20	Egg white protein	Y-αG + pNPG	>150.0	Yu et al. [20]
22	RVPSL	5	570.69	+1	9.75	60	Egg white protein	Y-αG + pNPG	>150.0	Yu et al. [20]
23	RVPSLM	6	701.88	+1	9.75	66.67	Egg white protein	Y-αG + pNPG	23.07	Yu et al. [20]
24	*GFPFYP	6	726.83	0	5.52	66.67	*Vaccaria hispanica*	Y-αG + pNPG	28^	Zheng et al.[40]
25	NVLQPS	6	656.74	0	5.52	50	Albumin	Y-αG + pNPG	100.0 ± 5.7	Yu et al. [10]
26	QITKPN	6	699.81	+1	8.75	33.33	Albumin	Y-αG + pNPG	>150.0	Yu et al. [10]
27	AEAGVD	6	560.56	-2	3.67	50	Albumin	Y-αG + pNPG	>150.0	Yu et al. [10]
28	LEPINF	6	731.85	-1	4.00	66.67	Albumin	Y-αG + pNPG	>150.0	Yu et al. [10]
29	ANENIF	6	706.75	-1	4.00	50	Albumin	Y-αG + pNPG	>150.0	Yu et al. [10]
30	AGLAPY	6	590.68	0	5.57	66.67	Egg white protein	Y-αG + pNPG	>150.0	Yu et al. [20]
31	DHPFLF	6	774.87	-1	5.08	66.67	Egg white protein	Y-αG + pNPG	>150.0	Yu et al. [20]

32	NEISFHA	7	816.87	-1	5.24	42.86	Synthetic	M-αG+ 4-MUG	No activity	Roskar et al.[23]
33	GHLYDDP	7	815.84	-2	4.20	28.57	Synthetic	M-αG + 4-MUG	No activity	Roskar et al.[23]
34	*CGHHHRDYC	9	1127.22	0	7.00	0	Synthetic	M-αG + 4-MUG	4991.68^	Roskar et al.[23]
35	*CTHYGFRGC	9	1043.18	+1	8.07	11.11	Synthetic	M-αG + 4-MUG	No activity	Roskar et al.[23]
36	RASDPLLSV	9	957.09	0	5.84	55.56	Egg yolk protein	Y-αG + pNPG	No activity	Zambrowicz et al. [29]
37	RNDDLNYIQ	9	1150.21	-1	4.21	22.22	Egg yolk protein	Y-αG + pNPG	No activity	Zambrowicz et al. [29]
38	YINQMPQKSRE	11	1393.58	+1	8.59	27.27	Synthetic	Y-αG + pNPG	1.21^	Zambrowicz et al.[30]
39	YINQMPQKSREA	12	1464.66	+1	8.59	33.33	Synthetic	Y-αG + pNPG	0.31^	Zambrowicz et al.[30]
40	VTGRFAGHPAAQ	12	1211.35	+1	9.73	50	Synthetic	Y-αG + pNPG	0.30^	Zambrowicz et al. [30]
41	LAPSLPGKPKPD	12	1219.45	+1	8.59	58.33	Egg yolk protein	Y-αG + pNPG	1065.6	Zambrowicz et al.[29]
42	YIEAVNKVSPRAGQF	15	1678.91	+1	8.59	46.67	Synthetic	Y-αG + pNPG	No activity	Zambrowicz et al. [30]
43	AGTTCLFTPLALPYDYSH	18	1970.23	-1	5.08	44.44	Egg yolk protein	Y-αG + pNPG	No activity	Zambrowicz et al. [30]

Physicochemical parameters for peptides (> amino acid residues) were computed using Expasy Bioinformatics Resource portal (http://web.expasy.org/protparam/)

[#]The physicochemical parameters of peptides (< 5 amino acid residues) were computed peptide property calculator available at http://www.biosyn.com/peptidepropertycalculatorlanding.aspx

Hydrophobicity was calculated using the peptide hydrophobicity/hydrophilicity analysis program at (http://peptide2.com/N_peptide_hydrophobicity_hydrophilicity.php)

* Cyclic peptide

^ These IC_{50} values were reported with other concentration units by the respective authors but were appropriately converted to their μM equivalents (in this study) for ease of comparison

Y-αG and pNPG refer to yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside respectively whileM-αG and 4-MUG refer to mammalian α-glucosidase and 4-methylumbelliferyl- α -D-glucopyranoside, respectively

of 39 out of the 43 peptides was assayed using the yeast α-glucosidase reaction and p-nitrophenyl glucopyranoside while in the case of the remaining 4 peptides,4-methylumbelliferyl-α-D-glucopyranoside was used as substrate. This allowed a more accurate comparison of IC_{50} values between peptides.

2.2 Effects of chain length, amino acid composition and sequence on the α-glucosidase inhibition

A tetrapeptide (STYV) was found to be the most potent α-glucosidase inhibitory peptide (IC_{50} = 0.012 µM) which was sequentially followed by peptides with 12 (VTGRFAGHPAAQ,IC_{50}= 0.30µM and YINQMPQKSREA, IC_{50}= 0.31µM),11 (YINQMPQKSRE, IC_{50}= 0.31µM) and 3 (PFP, IC_{50}= 8.62 µM) amino acid residues. On the contrary, complete lack of activity was reported for a dipeptide (PL) as well as peptides with 7 (NEISFHA and GHLYDDP), 9 (CTHYGFRGC, RASDPLLSV and RNDDLNYIQ), 15 (YIEAVNKVSPRAGQF) and 18 (AGTTCLFTPLALPYDYSH) amino acid residues (Table 1). These findings might suggest that there is no direct relationship between the chain length and the reported α-glucosidase inhibitory activities. In fact, this was further supported when correlation analysis was performed between the chain length and α-glucosidase inhibitory activity where a weak correlation (r^2 value of 0.104) was observed. It is also noteworthy that none of the peptides with 3-6 amino acid residues demonstrated complete lack of activity indicating that this range of chain length might be appropriate for designing moderately active α-glucosidase inhibitory peptides.

A close analysis of the amino acid composition of the five most potent α-glucosidase inhibitory peptides (STYV, YINQMPQKSREA, VTGRFAGHPAAQ, YINQMPQKSRE and PFP) indicated that these sequences contain at least an amino acid with hydroxyl group side chain(serine, threonine and tyrosine) and/or proline (Table 1). This was further supported by the observation of Zhang et al.[28]on silkworm pupae α-glucosidase inhibitory peptides where SQSPA had the best α-glucosidase inhibitory activity (IC_{50} = 20 µM) among all the tested peptides in the study. Findings on peptides derived from albumin [10] and egg white proteins [20]also suggested that the presence of proline and a basic amino acid (lysine and arginine) may be important for enhanced activity because RVPSLM, KLPGF and TPSPR displayed higher α-glucosidase inhibition than other peptides investigated in both studies. However, this may not necessary suggest that the presence of these amino acids is necessary for activity because some

peptides with at least one of these residues did not elicit α-glucosidase inhibition.[23, 29, 30]Although most of the peptides investigated for α-glucosidase inhibition did not contain methionine, Yu et al. [20]observed that RVPSLM (IC_{50} = 23.07 µM) had much higher activity than RVPSL (IC_{50} = > 150.0 µM) clearly indicating a requirement for methionine for enhanced activity. Moreover, two of the three most potent peptides (YINQMPQKSREA and YINQMPQKSRE) in this review contain a methionine residue. Based on the above observations, it is logical to suggest that serine, threonine, tyrosine, proline, arginine, lysine and methionine could be the relevant amino acids required for the α-glucosidase inhibitory activity of peptides. The lack of activity in the remaining few peptides containing either of these amino acids may be the result of the effects of other peptide variable(s).

The amino acid sequences of peptides have been demonstrated to be an important factor for their biological activities.[22, 26]Hence, α-glucosidase inhibition also seems to depend on the peptide sequence as the presence of hydroxyl group-containing amino acid residues (serine, threonine or tyrosine) or a basic amino acid (lysine orarginine) at the N-terminal end of the peptide appears to greatly influence the α-glucosidase inhibition (Table 1). This is because the peptides with relatively better activity (among all the peptides) such as STYV, YINQMPQKSREA, YINQMPQKSRE, RVPSLM, KLPGF, SQSPAand TPSPR had one of those amino acids at the N-terminal. On the other hand, the presence of proline has earlier been suggested to be an important requirement for the α-glucosidase inhibition activity but its optimal positioning within the peptide could not be systematically deduced. Suffice to say that most of the peptides with higher α-glucosidase inhibitory activity have proline residues closer to the C-terminal. So far, two amino acid residues (methionine and alanine) have been shown to greatly enhance the α-glucosidase inhibition when present at the C-terminal of the peptide. For methionine, RVPSLM (IC_{50} = 23.07 µM) had much higher activity than RVPSL (IC_{50} = > 150.0 µM) whilst for alanine, YINQMPQKSREA (IC_{50} = 0.31 µM) had approximately four fold higher activity than YINQMPQKSRE (IC_{50} = 1.21 µM) The foregoing observations suggest that either of these two amino acids (alanine or methionine) might be required at the C-terminal of highly active α-glucosidase inhibitory peptides.

Overall, based on the afore-mentioned analyses of the chain length, amino acid composition and sequence of peptides vis-à-vis α-glucosidase inhibitory activity, it is possible to

hypothesize that the requirements for optimal α-glucosidase inhibition of peptides are a sequence of 3 to 6 amino acid residues with either serine, threonine, tyrosine, lysine or arginine at the N-terminal and a proline residue closer to the C-terminal with a methionine or alanine occupying the ultimate C-terminal position. Indeed, this corroborated with computational modeling studies on bean α-glucosidase inhibitory peptides where the major interactions between α-glucosidase and the inhibitory peptides were hydrogen bonds and polar interactions with very little hydrophobic interactions.[31]

2.4 Effect of net charge, hydrophobicity and isoelectric point on the α-glucosidase inhibitory peptides

The net charge of the investigated peptides ranges from -2 to +1 but the ten most potent α-glucosidase inhibitory peptides had net charges of either 0 or +1 whilst for those peptides with no inhibitory activity, it ranges from -2 to +1 (Table 1). Moreover, the three peptides with a net charge of -2; EAGVD and AEAGVD [10] as well as GHLYDDP [23] had relatively low or no α-glucosidase inhibitory activity. Thus, unlike the high positive charge characteristics of other bioactive peptides such as antimicrobial peptides[13, 14], it appears that the glucosidase inhibitory peptides require a net charge of 0 or +1 for optimal activity or, perhaps, negative charge might diminish their activity.

Numerous studies have demonstrated the critical role of hydrophobicity for the activity of bioactive peptides such as DPP-IV and ACE inhibitory peptides.[32, 33] The correlation between hydrophobicity and α-glucosidase inhibitory activity (data from Table 1) showed a weak correlation (r^2 value of 0.0958).This is also supported by the observation that the most potent α-glucosidase inhibitory peptides had very few amino acids with bulky hydrophobic side chains (leucine, isoleucine and valine). Based on these observations, it could be deduced that the hydrophobic interactions may not be involved in the inhibition mechanism of α-glucosidase inhibitory peptides.

An extensive literature search has revealed that there is little or no study that attempts to decipher the possible relationship between α-glucosidase inhibition and the isoelectric points of peptides. This is in spite of the influence of isoelectric point on the activity of other bioactive peptides such as antioxidant and ACE inhibitory peptides. [34] The correlation analysis of α-glucosidase inhibitory activity and the isoelectric point of peptides revealed a very weak

correlation (r^2 value of 0.0056). The highly active and less active α-glucosidase inhibitory peptides have isoelectric point values across the acidic and basic range. It is therefore unlikely that the ability of peptides to inhibit α-glucosidase is dependent on the isoelectric point.

At present, it is difficult to make an exact prediction on the physicochemical requirements of α-glucosidase inhibitory peptides but from the available data, it appears that hydrophobicity and isoelectric point to serve as very poor predictors of α-glucosidase inhibitory activity of peptides. Extensive *in silico*and *in vitro* studies are required to address this important research question.

2.5 *In silico* simulated gastrointestinal digestion of α-glucosidase inhibitory peptides

For therapeutic applications, the α-glucosidase inhibitory peptides have to reach the intestinal lumen in an intact form to bind the α-glucosidase enzyme in mucosa of the small intestine. In fact, several strategies have been developed to improve the resistance of peptides to proteolytic digestion. One of these strategies is the formation of cyclic peptides and 3 of the identified peptides with α-glucosidase activity are cyclic peptides (Table 1). However, cyclization often involves complex chemistry and is often expensive[23],therefore small linear peptides are still the most cost effective and attractive design option. Among the 43 peptides evaluated for α-glucosidase inhibition, only 9 were resistant to GIT digestion by a combination of pepsin, trypsin and chymotrypsin as predicted by BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/en/biopep) database (Table 2). In fact, even the most potent peptide STYV (IC_{50} = 0.012 µM) was digested to STY.The highly potent peptides predicted to be stable to GIT digestion were SQSPA [28]and TPSPR [20]with IC_{50} values of 20 and 40.02µM respectively. Considering the activities and sequences of both peptides, minor structural and physicochemical changes may be needed to produce a highly active α-glucosidase inhibitory peptide that is resistant to GIT digestion. The ultimate economic benefit of such findings to the food and biopharmaceutical industries cannot be overemphasized.

On the other hand, BIOPEP prediction of the potential bioactivity of the digested fragments revealed that they mostly possess DPP-IV inhibitory activity (Table 2) in addition to prolyl endopeptidase and ACE inhibitory activity. Interestingly, it was previously noted that gastrointestinal digestion does not always lead to the complete loss of activity by peptides as evidenced in an *in vivo* study where oats-derived peptides retained anti-diabetic activity after oral

Table 2.*In silico* simulated gastrointestinal digestion of α-glucosidase inhibitory peptides and the predicted biological activity using the BIOPEP database

Peptide number	Peptide sequence	Released peptide(s)	Predicted biological activity of the released peptide(s)
1	CL	CL	None
2	YP	Y, P	None
3	VW	VW	DPP-IV inhibitor (VW)
5	PFP	PF, P	DPP-IV inhibitor (PF)
6	YPY	Y, PY	DPP-IV inhibitor (PY)
7	YPL	Y, PL	DPP-IV inhibitor (PL)
8	YPG	Y, PG	DPP inhibitor (PG), ACE inhibitor (PG), prolyl endopeptidase inhibitor (PG),
9	**STYV**	**STY**, V	None
10	YYPL	Y, Y, PL	DPP-IV inhibitor (PL)
11	QPGR	QPGR (not digested)	N/A
12	NSPR	NSPR (not digested)	N/A
13	QPPT	QPPT (not digested)	N/A
14	**SQSPA**	SQSPA (not digested)	N/A
15	TPSPR	TPSPR (not digested)	N/A
16	KLPGF	K, L, PGF	None
17	EVSGL	EVSGL (not digested)	N/A
18	EAGVD	EAGVD (not digested)	N/A
19	HAEIN	HAEIN	None
20	QIGLF	QIGL, F	None
21	DLQGK	DL, QGK	None
22	RVPSL	R, VPSL	None
23	RVPSLM	R, VPSL, M	None
24	*GFPFYP	GF, PF, Y, P	DPP-IV inhibitor (PF and GF)
25	NVLQPS	NVL, QPS	None
26	QITKPN	QITK, PN	DPP-IV inhibitor (PN)
27	AEAGVD	AEAGVD (not digested)	N/A
28	LEPINF	L, EPINF	None

29	ANENIF	ANENIF (not digested)	N/A
30	AGLAPY	AGL, APY	None
31	DHPFLF	DHPF, L, F	None
34	*CGHHHRDYC	CGHHHR, DY, C	Ion flow regulating peptide (DY)
38	YINQMPQKSRE	Y, INQMPQK, SR, E	None
39	YINQMPQKSREA	Y, INQMPQK, SR, EA	ACE inhibitor (EA)
40	VTGRFAGHPAAQ	VTGR, F, AGHPAAQ	None
41	LAPSLPGKPKPD	L, APSL, PGK, PK, PD	DPP IV inhibitor (PK)

Gastrointestinal simulation was conducted with a combination of chymotrypsin, trypsin and pepsin in the BIOPEP database (http://www.uwm.edu.pl/biochemia/index.php/en/biopep)

*Cyclic peptide
N/A means not applicable

treatment in diabetic rats [35]. Therefore, it is possible for some of these α-glucosidase inhibitory peptides to still serve a dual function of low to moderate inhibition of α-glucosidase and DPP-IV. This might be of great impact in the search of multifunctional anti-diabetic peptides [4, 29, 31]as peptides of longer sequences can be designed which could release fragments with α-glucosidase and DPP-IV inhibitory activities after GIT digestion.

2.6 Molecular docking studies

In the present review, it was found that the hydrophobicity and isoelectric point were poor predictors of inhibitory activity and to further evaluate the structural requirements for α-glucosidase inhibitory activity, three peptides were selected for detailed molecular docking analysis using Autodock Vina tool[27] via chimera version 1.11.2 (www.cgl.ucsf.edu/chimera/). The peptides were SQSPA that has high activity (IC_{50}= 20 µM) and was resistant to*in silico* GIT digestion, STYV that had the highest activity (IC_{50}= 0.012µM) but was digested to form STY with unknown bioactivity. Acarbose was included for comparative purposes.

Acarbose was found to have a binding energy of -7.6 kcal/mol with 3 hydrogen bond interactions (Table 3). SQSPA had a binding energy of -6.4 kcal/mol with 2 hydrogen bonds, STYV a binding energy -7.0kcal/mol) with 4 hydrogen bonds and STY with a binding energy of -7.2 kcal/mol and 6 hydrogen bonds(Figure 1). The lower IC_{50} and binding energy values, and two additional hydrogen bonds required for the interaction of STYV compared to SQSPA, indicated that hydrogen bond interactions are important for the inhibition. A. positive correlation (r^2 value of 0.668) was observed between the IC_{50} and binding energy values of the peptides suggesting that the *in vitro* and *in silico* data are in good agreement. It can therefore be predicted that the digestion product of STYV (STY) will have high α-glucosidase inhibitory activity.

The observed free binding energy values of the selected peptides indicated that the peptides have strong binding affinities with the C-terminal domain of human α-glucosidase which was comparable to that of acarbose. A number of synthetic α-glucosidase inhibitory compounds were reported to have higher free binding energy [36]compared to these peptides.The important binding site residues which are involved in the hydrogen bond interactions with the peptides are D1117, G1209, K1059, Y1062, N1480, E1640, I1716, K1625 and R1635. In the peptide ligands, the N-terminal residue (S1) seems to be highly involved in the hydrogen bond formation (Figure 1) which further supports our earlier observation that the presence of a hydroxyl group containing

Table 3. Docking results and binding free energy (kcal/mol) of acarbose and the selected peptides with the C-terminal of human α-glucosidase

Peptide ligand	Binding energy (kcal/mol)	Number of hydrogen bonds	Interacting residue of the peptide	Interacting residue of the α-glucosidase	Hydrogen bond distance (Å)
Acarbose	-7.6	3	-	-	-
SQSPA	-6.4	2	S1	D1117	2.410
			S1	G1209	2.488
STYV	-7.0	4	S1	G1209	2.509
			T2	K1059	2.733
			S1	Y1062	2.137
			Y3	N1480	2.267
STY	-7.2	6	S1	E1640	2.409
			Y3	I1716	2.427
			T2	K1625	2.187
			Y3	R1635	2.077
			Y3	R1635	2.206
			Y3	R1635	1.972

residue at the N-terminal is crucial for the α-glucosidase inhibitory activity of peptides. Indeed, the observation of a lower binding energy coupled with more hydrogen bond interactions of STY (a tripeptide composed of 3 hydroxyl group containing amino acid residues) also supports our earlier claim as well as the findings of Mojica and de Majia[31] where serine was the principal amino acid residue interacting with α-glucosidase.

3. CONCLUSION AND FUTURE PERSPECTIVES

This article has identified peptides as promising therapeutic molecules for the inhibition of α-glucosidase activity. Identified structural requirements are tri – to hexapeptides with serine, threonine, tyrosine, lysine or arginine as the ultimate N-terminal residue and proline preferably at the penultimate C- terminal position while alanine or methionine at ultimate C-terminal position. No specific requirements related to peptide hydrophobicity and charge were identified. Future design of α-glucosidase inhibitory peptides could therefore take these features into cognizance to enable the discovery of therapeutically active α-glucosidase inhibitory peptides. Unfortunately, most of the highly or moderately active peptides (observed in this review) with the above structural features were hydrolyzed during simulated GIT digestion with the exception of SQSPA which makes this peptide very attractive for future and detailed α-glucosidase inhibition studies. Although the most potent peptide observed in this review STYV was also digested, it could still be subjected to detailed further studies because molecular docking studies have predicted an even better α-glucosidase inhibitory activity of the resulting product of its gastrointestinal digestion (STY).

In spite of the attempt in this article to deduce the basic structural requirements for α-glucosidase inhibition by peptides, it is possible to suggest that a more detailed quantitative structure activity relationship study should be conducted in order to more precisely decipher the key features required for activity. Furthermore, detailed mechanistic studies are needed to determine the exact mechanism of α-glucosidase inhibition of the peptides. The combination of the detailed quantitative structure activity relationship and mechanistic studies would certainly facilitate the rational design of effective α-glucosidase inhibitory peptides as antidiabetic drugs.

In the search of α-glucosidase inhibitors, it is usually of great pharmaceutical interest to investigate the corresponding α-amylase inhibitory effects because most of the side effects associated with the currently available α-glucosidase inhibitors are linked to excessive inhibition of α-amylase activity. However, most of the α-glucosidase inhibitory peptides were not investigated for the α-amylase inhibition. Apart from studies on peptides from albumin, *Aspergillus awamori*, egg white and black bean proteins, no other fully sequenced peptide was investigated for α-amylase inhibition. Therefore, this is an area that deserves utmost research attention if pharmaceutically relevant α-glucosidase inhibitors are to be developed from bioactive peptides.

An important potential application of α-glucosidase inhibitory peptides is to serve as components of nutraceuticals or biopharmaceuticals and not necessarily as conventional drugs. However, relevant and more practical studies around this research area are completely missing in the present literature which suggests the need to refocus scientific attention to this potentially area. Furthermore, studies to ascertain possible food-drug interaction may be needed to make a definite conclusion on the potentials of these peptides as nutraceuticals.

Another pertinent observation in this article is that no study investigated the effects of α-glucosidase inhibitory peptides in animal models of diabetes. Hence, there is a dearth of information on the *in vivo* α-glucosidase inhibitory effects of these peptides which underscores the need to also refocus research efforts to this area so as to possibly pave the way for further clinical trials. However, the review highlights the importance of computational analysis prior to the implementation of such studies as beneficial effects may be due to the digested peptide having altered activity as observed for STYV or that the peptides target other enzymes such as DPP-IV.

LIST OF ABBREVIATION

ACE = Angiotensin Converting Enzyme

IDF = International Diabetes Federation

T2D = Type 2 diabetes

DPP-IV= Dipeptidyl peptidase IV

GIT = Gastrointestinal tract

CONFLICT OF INTEREST

The authors confirm that the article has no conflict of interest

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