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ORIGINAL ARTICLE

Quantitative structure–activity relationship modeling of renin-inhibiting dipeptides

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Abstract Partial least squares regression method was used to analyze a peptide dataset and construct inhibitory models for renin-inhibitory natural dipeptides. The models were computed with the renin-inhibitory activity as dependent variable (*Y*) and the peptide structural properties as predictors (*X*); validation was conducted using cross-validation and permutation tests. The amino acid descriptors were based on the *3*- and *5*-*z* scales of 20 coded amino acids to produce models that explained 71.6% of *Y* with a 33.8% predictive ability and 75.2% of *Y* with a predictive power of 50.8%, respectively. In both models, low molecular size amino acids with hydrophobic side chains were preferred at the N-terminus, while amino acids with bulky side chains were preferred at the C-terminus for potency. Based on the *5*-*z* model, four Trp (W)-containing antihypertensive dipeptides (IW, LW, VW and AW) were predicted as the most potent renin inhibitors. The peptides were synthesized and in vitro inhibition assay showed that IW and LW inhibited 70% (IC_{50}, 2.3 mM) and 37% renin activity at 3.2 mM, respectively, whereas VW and AW were inactive. There was no correlation between the observed renin-inhibitory activities and angiotensin-converting enzyme inhibitory activities of the dipeptides. We concluded that the structural similarities between isoleucine and leucine could have contributed to their distinct inhibitory activity when compared to alanine and valine. Therefore, IW may be a useful template for the development of advanced forms of highly active low molecular size antihypertensive peptides and peptidomimetics.

Keywords Renin inhibitors · Dipeptides · Quantitative structure–activity relationship (QSAR) · Partial least squares regression (PLS) · Amino acid descriptors

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Introduction

The renin–angiotensin system (RAS) has provided key molecular targets for therapeutic agents toward the treatment and management of hypertension, a contributing risk factor to human cardiovascular diseases and a major public health concern especially in the Western world. This is primarily due to the role of the RAS pathway in controlling blood pressure through the production of angiotensin (AT)-II, a potent vasoconstrictor, from AT-I by the action of angiotensin I-converting enzyme (ACE). Consequently, ACE inhibitors have been widely used to control blood pressure during hypertension, and several food protein-derived peptides have shown both ACE-inhibitory and antihypertensive activities (Aluko 2008). Another important molecular target for antihypertension drug discovery is the renin-catalyzed conversion of angiotensinogen to AT-I, which is the rate-limiting step of RAS (Staessen et al. 2006). The inhibition of renin activity (RA) can provide better blood pressure control during hypertension than ACE inhibition since it prevents the formation of AT-I, which can be converted to AT-II in some cells via an ACE-independent chymase-catalyzed reaction (Staessen et al. 2006; Segall et al. 2007). Moreover, since angiotensinogen is the only known renin substrate, targeting renin in the RAS pathway ensures high specificity in controlling

elevated blood pressure as opposed to ACE inhibition, which can also affect other biochemical pathways leading to toxic effects (Acharya et al. 2003; Staessen et al. 2006). Several synthetic peptides and non-peptides have been reported to possess renin-inhibitory activity but their application as orally active antihypertensive agents has been hindered due to poor pharmacokinetic attributes (Rahuel et al. 2000; Fischer and Hollenberg 2001; Wood et al. 2003). Till date, aliskiren is the only known clinically relevant non-peptide renin-inhibiting antihypertensive agent (Sepehrdad et al. 2007); current research efforts are continuously directed toward the discovery of potent non-toxic renin inhibitors. Recent studies have shown that food protein-derived peptides and enzymatic hydrolysates possess the ability to inhibit RA in vitro (Udenigwe et al. 2009; Li and Aluko 2010). These research efforts could lead to the discovery of a new generation of cheaper renin inhibitors with no side effects, since the peptides were generated from food proteins. Moreover, knowledge of the structure–function property of these food-derived peptides can lead to the design and synthesis of more potent renin-inhibiting antihypertensive agents, especially peptidomimetics. To the best of our knowledge, there is scanty information in the literature on the structural requirements for renin-inhibiting food-derived peptides that contain only coded amino acids.

Quantitative structure–activity relationship (QSAR) modeling is a practical and reliable method in chemometrics for studying the relationship between molecular structures of therapeutic agents and biological activities. In peptide QSAR, statistical multiple regression analysis such as partial least squares (PLS) projection of latent structure have been widely used to develop models that relate the molecular structures to variation in the biological activities. In other words, bioactivity data can be modeled as a function of molecular structures of peptides (Sandberg et al. 1998; Wu et al. 2006a). Using amino acid *z* scale descriptors, PLS has been successfully applied in developing good models in QSAR study of various bioactive peptides (Hellberg et al. 1987) including models that can explain the structural requirements for ACE-inhibiting peptides of various chain lengths (Wu et al. 2006a, b). QSAR models for peptides' bitterness property (Wu and Aluko 2007) and for the study of the functional properties of polypeptides (Siebert 2003) have also been reported.

The objectives of this study were to (a) develop QSAR models to explain the structure–activity relationship of a group of natural renin-inhibiting dipeptides, (b) use the models to predict potent renin-inhibiting peptides followed by in vitro evaluation of their bioactivity, and (c) evaluate the ACE-inhibitory activity of the most potent predicted peptides in order to determine if there is a similarity between the structural requirements for ACE and renin inhibition.

Methods

Peptide dataset

The peptide dataset consisted of 13 renin-inhibiting dipeptides that were originally identified in our lab from mass spectrometry analysis of an enzymatic pea (*Pisum sativum*) protein hydrolysate (Table 1). The renin-inhibitory activities (RI, %) were measured at 3.2 mM peptide concentration and were log-transformed prior to modeling. Amino acid sequences of the peptides, RI and log RI are presented in Table 1.

PLS regression modeling

The peptide QSAR was carried out using PLS analysis as previously reported for other bioactive peptides (Wold et al. 2001; Wu et al. 2006a, b). This approach to peptide QSAR employs the amino acid *3-z* scale and the extended *5-z* scale to describe the structural features of the amino acid components of the dipeptides, and this constitutes the multivariate peptide descriptor (*X*) matrix. Due to the wide range of data for the pea protein-derived dipeptides, bioactivity data were expressed as log RI and this constitutes the dependent variable (*Y*) matrix. In order to ensure consistency in data interpretation, both the *3-z* scale and the *5-z* scale amino acid descriptors used in this study were reported by the same research group (Hellberg et al. 1987; Sandberg et al. 1998), and the same approach was also used for the bioactivity data (Li 2010). The *3-z* scale (*z1*, *z2*, *z3*) of the amino acids was previously calculated by principal component analysis from a matrix that consisted of 29 physicochemical variables of each of the 20 coded amino acids including molecular weight, ^{1}H- and ^{13}C-NMR data,

Table 1 Dipeptide dataset with in vitro renin-inhibitory activity (RI) at 3.2 mM peptide concentration and log RI of the dipeptides

No.	Peptide sequence	RI (%)	log RI
1	IR	49.11	1.69
2	LR	33.97	1.53
3	NR	25.36	1.40
4	KF	28.78	1.46
5	EF	22.36	1.35
6	QF	12.04	1.08
7	RF	6.35	0.80
8	SF	15.90	1.20
9	YA	15.13	1.18
10	FK	8.92	0.95
11	FE	1.81	0.25
12	FQ	8.64	0.93
13	FT	20.40	1.31

pK_a, p*I*, substituent van der Waals volume, and so on. The *z* scales are interpreted to be related to hydrophilicity (*z1*), steric properties or side chain bulk/molecular size (*z2*) and electronic properties (*z3*) (Hellberg et al. 1987), whereas the additional descriptors (*z4* and *z5*) in the *5-z* scale, which are difficult to clearly interpret, are related to heat of formation, and "hardness" and electrophilicity, respectively (Sandberg et al. 1998). The amino acid at the N-terminus of the dipeptides was designated as *n1* and its structural properties were described as *n1z1*, *n1z2* and *n1z3* for the *3-z* scale or *n1z1*, *n1z2*, *n1z3*, *n1z4* and *n1z5* for the *5-z* scale (Wu et al. 2006a, b). Likewise, the amino acid at the C-terminus was designated as *n2* and its properties were described as *n2z1*, *n2z2* and so on. The relationship between the renin inhibition data (*Y*) and the peptide structural properties (*X*) was modeled by PLS using SIMCA-P software version 11.0 (Umetrics AB, Umeå, Sweden). All variables were centered and scaled to unit variance to ensure equal contribution in the models. The models were theoretically validated using a combination of cross-validation and permutation tests as previously described (Wold and Eriksson 1995; Wold et al. 2001; Wu et al. 2006a).

Peptide synthesis

All 13 peptides in the dataset (Table 1) as well as the most potent predicted peptides (IW, LW, AW and VW) were synthesized by Genscript Corp. (Piscataway, NJ, USA). The purity (95–99%) of each peptide was measured by high-performance liquid chromatography (HPLC) and their structures were confirmed by mass spectrometry (Genscript Corp.).

Renin inhibition assay

Renin inhibition assay was conducted by fluorescence spectrometry using the Renin Inhibitor Screening Assay Kit (Cayman Chemicals, Ann Arbor, MI, USA) as previously described (Udenigwe et al. 2009). Briefly, the assay mixture contained 10 μM of renin substrate, human recombinant renin and the peptide sample in 50 mM Tris–HCl buffer containing 100 mM NaCl (pH 8.0). The renin substrate and peptide samples were mixed and pre-warmed to 37°C for 10 min prior to the assay followed by the addition of renin to the mixture to initiate the reaction. The increase in fluorescence intensity was measured for 10 min at 37°C. The spectrofluorometer was set at excitation wavelength of 340 nm, emission wavelength of 490 nm, excitation bandwidth of 5 nm and emission bandwidth of 10 nm. RA was expressed as fluorescence intensity unit per min and inhibitory activity (%) of the peptides was calculated as $\{[(RA_{(blank)} - RA_{(sample)})/RA_{(blank)}] \times 100\}$, where $RA_{(blank)}$ and $RA_{(sample)}$ are the renin reaction rates in the absence and presence of the peptide samples, respectively.

ACE inhibition assay

The predicted peptides were also evaluated for ACE inhibition in order to investigate if there are similarities in the structural requirements of the peptides for renin and ACE inhibition. The ACE inhibition assay was conducted as previously reported using *N*-(3-[2-furyl]acryloyl)-phenylalanylglycyl-glycine (FAPGG) as ACE substrate (Udenigwe et al. 2009). The activities of the dipeptides were reported as the concentration that resulted in 50% inhibition of ACE activity (IC_{50}), which was calculated from non-linear regression analysis of a plot of ACE inhibition (%) versus peptide concentration.

Statistical analysis

The enzyme inhibition data were reported as mean ± standard deviation of two or three replicated assays and all the data were subjected to one-way analysis of variance (ANOVA). Statistical significance of differences was evaluated by Duncan's multiple range test ($P = 0.05$) using the Statistical Analysis Systems (SAS) software version 9.2 (SAS Institute, Cary, NC, USA).

Results and discussion

QSAR of renin-inhibitory dipeptides by PLS

Low molecular size peptides, especially di- and tripeptides, with biological properties are excellent therapeutic candidates since they are usually resistant to gastrointestinal proteolysis and can be absorbed intact into blood circulation to sites where physiological activity is needed (Mathews and Adibi 1976; Roberts et al. 1999; Vermeirssen et al. 2004). Thus, peptides containing only two amino acid residues were chosen for this QSAR study in order to increase the physiological relevance of the resulting predicted potent renin-inhibiting peptides. However, it should be noted that binding is a matter of stereoelectronic complementary features between ligand and receptor; therefore, the dipeptides may not necessary act in a similar way to the two enzyme substrates, angiotensinogen and angiotensin I. The dipeptides used in the dataset were originally identified from a renin-inhibiting pea protein hydrolysate fraction, and these peptides individually showed varying renin-inhibitory activities (Table 1). The unique structural properties (*X*) and the wide range of bioactive properties (*Y*) of the peptides

were used to develop QSAR models to explain the relationship between *X* and *Y*, and also to predict novel peptides with enhanced renin-inhibitory activity. The first modeling of the *3-z* scale amino acid descriptors with *Y* resulted in a three-component PLS model that explained 35.6% of the sum of squares in *Y*-variance with a predictive ability of 10.5% of the dipeptides, whereas the *5-z* scale provided a one-component model that could explain 46.2% of *Y* with a predictive power of 9.53% (derived from cross-validation coefficient, Q^2_{cv}). The *t1/u1* (*tu*) PLS scores plots, which shows the relationship between *X* and *Y*, revealed the presence of two outliers (Nos. 9 and 13 in Table 1) in these models, which were removed from the peptide dataset (Wold et al. 2001). Subsequently, the new peptide dataset was used to construct second models in an attempt to increase their predictive powers. The summary of these new PLS models is shown in Table 2. The second modeling resulted in improved models: (1) a one-component model based on the *3-z* scale descriptors that could explain 71.6% of the *Y* sum of squares (predictive ability of 33.8%) and (2) another one-component PLS model based on the *5-z* scale descriptors that explains 75.2% of the renin inhibition data with 50.8% predictive ability of the peptide dataset. These models are illustrated in Fig. 1, which shows the relationship between the observed and predicted renin-inhibitory activity (log RI) of the peptide dataset. The multiple correlation coefficients (R^2) of the models were higher than 0.70 with the *5-z* scale model showing higher value than the *3-z* scale model. Thus, the robustness of the model was substantially improved when the *5-z* scale was used compared to the *3-z* scale, which is in agreement with previous QSAR studies of ACE-inhibiting tetra- and penta-peptides (Wu et al. 2006b) and protein functional properties (Siebert 2003).

One approach used to validate QSAR models is to use part of the dataset as training set in developing the model and the other part as test sets in testing the predictive capability of the model (Hellberg et al. 1987; Wold et al. 2001). This approach was not applied to this present work due to the limited number of observations. Instead, the *z* scale-based PLS models were validated initially by cross-validation during modeling and their predictive power also validated by permutation, where the *Y* data were each randomly permuted a number of times but with unaltered *X*-variable followed by a QSAR modeling of each permutation (Wold and Eriksson 1995). Twenty permutation rounds yielded cumulative R^2 (R^2_{cum}) and Q^2_{cv} intercept values of 0.181 and −0.093 for the *3-z* scale, and 0.191 and −0.177 for the *5-z* scale, respectively (Table 2). R^2_{cum} and Q^2_{cv} intercepts are measures of model fit and it was suggested that R^2_{cum} intercept < 0.3 and Q^2_{cv} intercept < 0.05 constitute a desirable limit for a valid model (Sandberg et al. 1998; Andersson et al. 1998). Thus, the R^2_{cum} and Q^2_{cv} intercept values observed for these two models indicate that the models can be considered valid and could not have resulted by chance. These models are regarded as good models considering that the modeling was based on random peptide dataset that were not statistically designed.

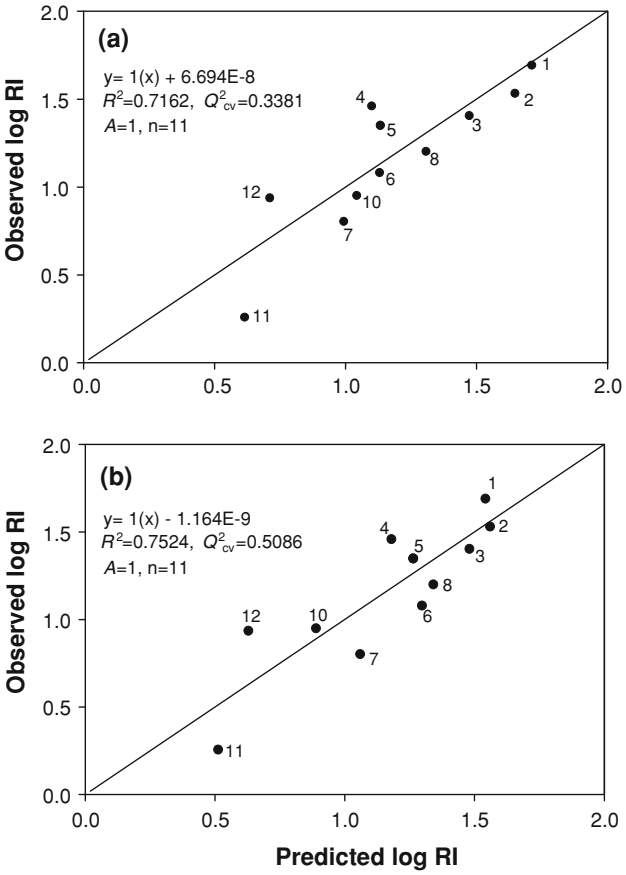

Fig. 1 Relationship between the observed and the predicted values of log RI using the amino acid **a** *3-z* scale and **b** *5-z* scale

Table 2 Summary of the PLS models using the amino acid *3-z* scale and *5-z* scale

Model	*N*	*A*	R^2Y	Q^2_{cv}	Intercept R^2_{cum}	Intercept Q^2_{cv}	RMSEP
3-z scale	11	1	0.7161	0.3381	0.181	−0.093	0.1981
5-z scale	11	1	0.7524	0.5086	0.191	−0.177	0.1929

Multiple correlation coefficient (R^2) indicates the sum of squares of *Y* explained and estimate of model fit, whereas the cross-validated correlation coefficient (Q^2_{cv}) indicates the model's predictive ability

N number of peptides in the dataset, *A* number of significant components, *RMSEP* root mean square error of prediction

Peptide prediction

The models were used to determine structural properties of the dipeptides that contribute substantially to renin inhibition, and were subsequently applied in predicting peptides with putatively enhanced activity. The PLS coefficient plot shows the extent of contribution of the peptide structural descriptor (*X*) to modeling of the bioactive property (Fig. 2). The contribution of an *X*-variable in the models depends on the coefficient value relative to the origin in the loading space (Wold et al. 2001), i.e. the higher the coefficients in both directions, the more the contribution of the *X*-variable in explaining or predicting *Y*, and the sign indicates the direction of the relationship (Sandberg et al. 1998). The coefficient plot of the *3-z* scale model (Fig. 2a) shows that *n1z1* and *n2z2* are positively correlated to *Y*, whereas *n2z1*, *n1z2*, *n1z3* and *n2z3* are negatively correlated to bioactivity. This pattern was also observed for the *z1–z3* descriptors in the *5-z* scale model, whose coefficient plot also indicated a positive relationship of renin inhibition (*Y*) with *n2z4* and *n1z5*, and inverse correlation with *n1z4* and *n2z5* (Fig. 2b). In order to determine the importance of the variables in terms of relative contributions of the descriptors (*X*) in modeling both *Y* and *X*, the variable importance for the projection (VIP) plots were obtained for the models. A VIP value that is greater than one indicates an important *X*-variable with above average contribution while VIP values of less than 0.5 indicate unimportant peptide descriptors; values between 0.5 and 1 could be important or not depending on the size of the dataset (SIMCA-P 11 Software Analysis Advisor 2005). For this work, *X* is regarded as important contributor only when its VIP > 1. The VIP plots indicate that *n2z2* and *n1z2* are very important descriptors in both models, in addition to *n2z4* and *n1z5* contributions to the *5-z* scale model (Fig. 3); this also shows the contributions of *z4* and *z5* to model quality. Based on the coefficient and VIP plots of these PLS models, it could be observed that steric properties or side chain bulk/molecular size (*z2*) of the amino acid plays the most important role in determining the potency of the peptides as renin inhibitors regardless of the location of the amino acid residue on the

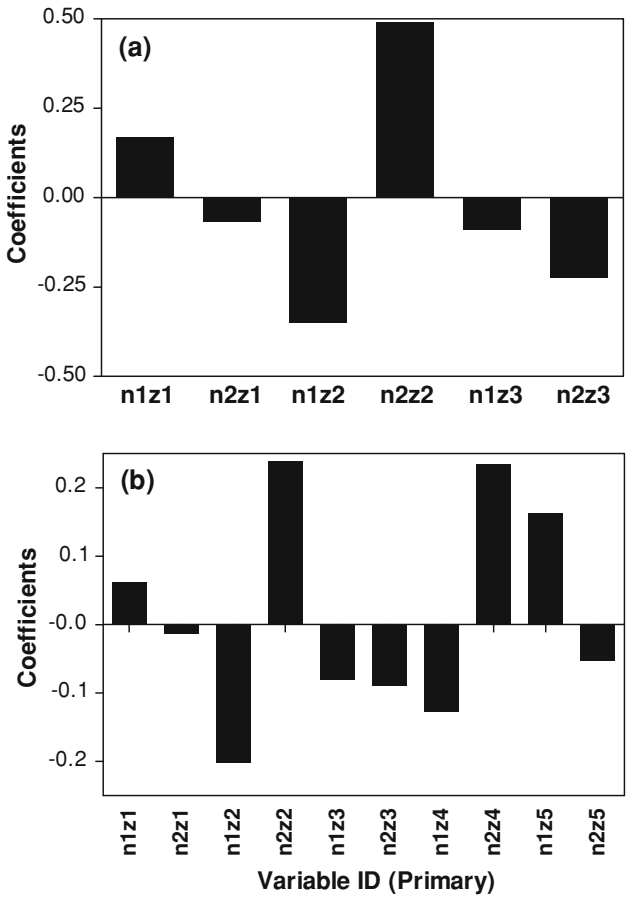

Fig. 2 PLS regression coefficients of the **a** *3-z* scale and **b** *5-z* scale of dipeptides; the contribution of an *X*-variable in the models depends on the coefficient value relative to the origin

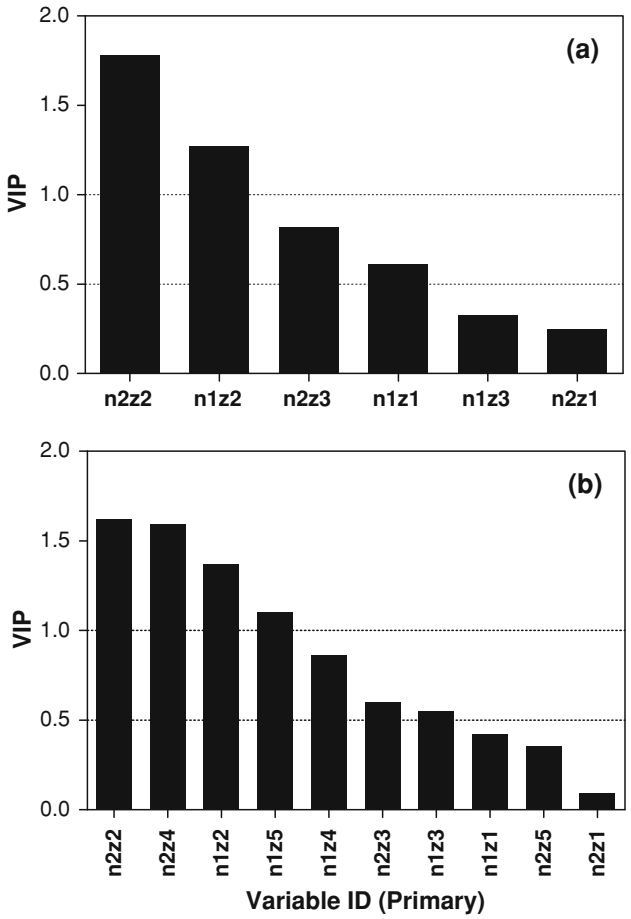

Fig. 3 Variable importance for the projection (VIP) of the **a** *3-z* scale and **b** *5-z* scale models

dipeptide; thus, both *n1* (N-terminus) and *n2* (C-terminus) of the peptides are equally important. New models that excluded unimportant *X*-variables with VIP < 0.5 resulted in improved model quality and predictive abilities of the PLS models, especially the *5-z* scale model ($N = 11$, $A = 1$, $R^2Y = 0.777$, $Q^2_{cv} = 0.614$) and *3-z* scale model ($N = 11$, $A = 1$, $R^2Y = 0.665$, $Q^2_{cv} = 0.446$).

According to the old and new models, bulky or high molecular size amino acid residues decrease the renin-inhibitory property of a dipeptide if the amino acid is located at the N-terminus of the peptide, whereas the same amino acids could substantially increase the potency of the peptide if located at the C-terminus. In addition, the N-terminus amino acid residue must be highly hydrophobic for increased renin inhibition by the dipeptide. Based on the PLS regression coefficients (Fig. 2), electronic properties of the amino acid residues at both terminals of the dipeptides are negatively correlated to log RI. Accordingly, these amino acids should be non-polar for increased potency, even though their contributions are regarded as less important in the VIP plot (Fig. 3b). Therefore, in order to observe potent renin-inhibitory activity, these models showed that amino acids Val, Leu, Ile and Ala (↑*z5*, ↓*z2*, ↓*z3*) are preferred at the N-terminal, whereas Trp, Tyr and Phe (↑*z2*, ↑*z4*, ↓*z3*, ↓*z5*) are preferred at the C-terminal of dipeptides. Consequently, different combinations of these amino acids yielded 12 dipeptides with a range of predicted log RI of 1.17–1.98 for the *3-z* scale model and 1.33–1.91 for the *5-z* scale model, which translate to 15.0–96.5 and 21.7–81.0% renin-inhibitory activity, respectively, at 3.2 mM peptide concentration. It was observed that the most potent predicted peptides all possess Trp (W) at the C-terminus and include VW, AW, IW and LW with predicted renin inhibition of 96.5, 82.2, 72.5 and 62.5%, respectively, for the *3-z* scale model and 71.1, 81.2, 66.2 and 69.0%, respectively, for the *5-z* scale model, at 3.2 mM peptide concentration. Conversely, peptides with C-terminus Phe showed the weakest predicted renin inhibition compared to those with C-terminus Trp or Tyr. The most potent Trp-containing peptides were synthesized for confirmation of the predicted activities and for testing of the robustness of the models.

Renin inhibition by predicted dipeptides

In vitro enzyme inhibition assay using human recombinant renin showed that dipeptides IW and LW inhibited 70 and 37% of RA at 3.2 mM peptide concentration, respectively, whereas VW and AW were inactive under the same assay conditions. The activity observed for IW was similar to the values predicted using the two PLS models but that of LW was lower than the predicted values (Table 3). In addition,

Table 3 Predicted and observed log RI at 3.2 mM peptide concentration for the predicted renin-inhibiting dipeptides and the prediction errors

Peptide sequence	log RI				
	Predicted		Observed	Prediction error	
	3-z scale	*5-z* scale		*3-z* scale	*5-z* scale
IW	1.86	1.82	1.84	−0.02	0.02
LW	1.79	1.83	1.57	−0.22	−0.26
AW	1.91	1.91	0.00	−1.91	−1.91
VW	1.98	1.85	0.00	−1.98	−1.85

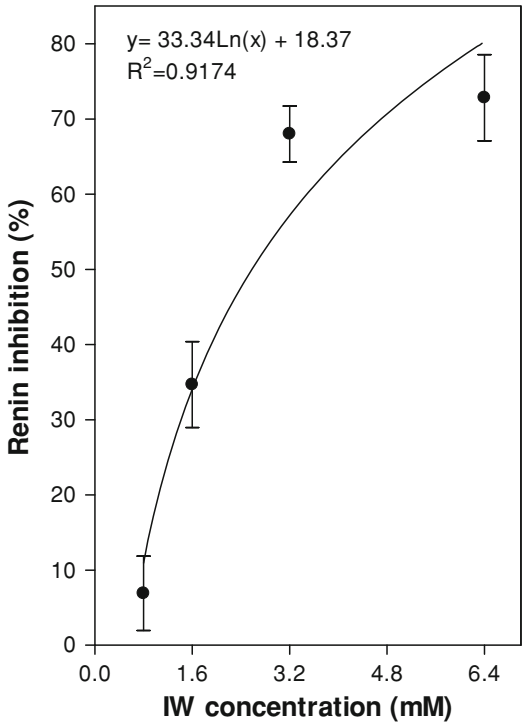

Fig. 4 Concentration-dependent in vitro inhibition of human recombinant renin by dipeptide IW with IC_{50} value of 2.32 ± 0.07 mM

Fig. 4 showed that IW displayed a concentration-dependent inhibition of RA with 50% inhibitory concentration (IC_{50}) value of 2.3 ± 0.07 mM (0.72 ± 0.02 mg/ml). Although the most potent predicted peptide (IW) was not as active as high molecular size peptide and non-peptide renin inhibitors (Fischer and Hollenberg 2001; Rahuel et al. 2000; Staessen et al. 2006; Wood et al. 2003), possible efficient absorption across the enterocytes due to size and resistance to gastrointestinal proteolysis in vitro (Enari et al. 2008) could encourage its potential use as oral anti-hypertensive agent. Moreover, IW was found to be more active than all the dipeptides in the original dataset used to develop the PLS models. Thus, the *z* scale models reported in this study were relevant toward the discovery of a new

renin-inhibiting dipeptide. Conversely, the PLS models did not correctly predict the renin-inhibitory potential of dipeptides VW and AW, which showed high prediction errors (Table 3). It is important to note that the bioactive predicted dipeptides contain branched-chain aliphatic amino acid isomers, Leu or Ile, at the amino end of the peptides contrary to the inactive peptides that possess N-terminal Val or Ala. Consequently, the pattern of renin inhibition by the dipeptides correlated with hydrophobicity of the N-terminal amino acids where Ile > Leu > Val > Ala (*z1* values of −4.44, −4.19, −2.69 and 0.07, respectively). This observation confirms the need for the presence of a highly hydrophobic, low molecular size N-terminal amino acid in renin-inhibiting dipeptides, as suggested by the PLS models. Moreover, since the N-terminal hydrophobic amino acids possess different conformations that affect their interaction with the enzyme, the lack of contribution of peptide conformational state to the derived principal properties (Dunn and Wold 1995) also constitutes a limitation in the predictive power of the *z* scale-based PLS models.

Relationship between renin and ACE inhibition

Since there are similarities between the predicted structural requirements for renin inhibition reported in this study and predicted requirements for ACE inhibition by dipeptides (Cheung et al. 1980; Wu et al. 2006a), the renin inhibition data were compared to the ACE-inhibitory activities of the synthesized dipeptides. Literature data on the ACE-inhibitory activities of the four predicted dipeptides were inconsistent; a wide range of log IC_{50} were reported for these peptides (Wu et al. 2006a), which translate to IC_{50} values of 1.4–10.8 μM for VW, 10–18.6 μM for AW, 1.5–12.4 μM for IW and 6.7–50.1 μM for LW. Due to this inconsistency, the dipeptides were evaluated for ACE inhibition in our laboratory under the same assay conditions. This present study confirmed that the dipeptides inhibited ACE activity in concentration-dependent fashions with IC_{50} values of 4.74 μM for IW, 7.1 μM for VW, 34.8 μM for AW and 38.9 μM for LW. Table 4 shows that this trend of ACE inhibition by the dipeptides is in agreement with a previous report (Sato et al. 2002) and the activity of IW is exactly the same as reported by Ono et al. (2006). Based on these data and the aforementioned literature information, there was no observed relationship between the renin and ACE-inhibitory activities of the dipeptides. Moreover, IW was found to be generally more active than the other Trp-containing dipeptides in inhibiting both enzymes of the RAS pathway. All the predicted dipeptides have been previously shown to lower blood pressure in spontaneously hypertensive rats (Fujita et al. 2000; Sato et al. 2002; Nii et al. 2008). The in vivo antihypertensive effect of IW was observed to be more pronounced than the other dipeptides; it reduced systolic blood pressure (SBP) by 6.3% (−14.6 mmHg) and 6.4% (−13.8 mmHg) at 0.1 and 1 mg/kg body weight (BW), respectively, after 9 h of administration (Sato et al. 2002), and in another study, by 22 mmHg at 60 mg/kg BW (Fujita et al. 2000). Therapeutic agents with dual effects as ACE and renin inhibitors can provide better blood pressure-lowering effects during hypertension than compounds that inhibit only ACE activity. Thus, the combined moderate renin inhibition and strong ACE inhibition displayed by IW may have contributed to its effects in SHR leading to pronounced blood pressure-lowering activity.

Table 4 ACE-inhibitory activities of the Trp (W)-containing dipeptides

Peptide sequence	IC_{50} (μM)		
	A	B	C
IW	4.74 ± 0.04	4.70	1.50
LW	38.92 ± 1.97	17.40	23.60
AW	34.86 ± 1.42	6.40	18.80
VW	7.08 ± 0.08	2.50	3.30

A data from the present study using FAPGG as ACE substrate, *B* data adapted from Ono et al. (2006), *C* data adapted from Sato et al. (2002). B and C used hippuryl-L-histidyl-L-leucine (HHL) as ACE substrate

This is the first QSAR study toward elucidation of structural requirements for renin inhibition by food protein-derived peptides using the *z* scale amino acid descriptors. Based on these models, we concluded that hydrophobic amino acid residue at the N-terminus (e.g. Ile) and bulky amino acid at the C-terminus (e.g. Trp) contribute to the potency of renin-inhibiting dipeptides. The fact that only IW and LW were effective against renin suggests that the required configuration for inhibition, especially in terms of amino acid conformation and spatial characteristics, is very specific since these peptides have the same size but are isomers. The high activity of IW suggests that it may serve as a useful template for developing more efficient renin-inhibitory peptides and peptidomimetics. However, future work must be based on 3D QSAR which can give information on the conformational factors that contribute to activity of the inhibitory peptides. The use of a larger dipeptide dataset derived from experimental design along with docking studies could also contribute to successful elucidation of the peptide configuration needed for inhibitory activity.

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