

TOWARDS BOVINE CONNECTIVE TISSUE UTILIZATION: *IN SILICO* INSIGHT ON THE POTENTIAL OF COLLAGEN IN PRODUCTION OF ACE INHIBITORY PEPTIDES

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Abstract – Bovine collagen was systematically assessed as a potential precursor of ACE inhibitory peptides based on *in silico* analysis. The sequence alignments of bovine collagen alpha-1(I) and collagen alpha-2(I) by BLAST analysis revealed relatively high sequence homology (65%). BIOPEP analyses indicated that *in silico* proteolysis theoretically released 782 and 775 potential ACE inhibitory peptides from alpha-1(I) and alpha-2(I) collagen, respectively. Papain was the most effective protease to release the most ACE inhibitory peptides. Two QSAR models by PLS regression analysis for di- and tri-peptides were established on the basis of reported literature. The ACE inhibitory activities of collagen-derived peptides were further predicted; a number of potent ACE inhibitory peptides with low IC₅₀ values were ascertained.

Key Words – bovine collagen; ACE inhibitory peptides; *in silico* analysis; BIOPEP database; QSAR

I. INTRODUCTION

Hypertension, a main risk factor responsible for cardiovascular diseases, threatens estimated a billion people (14.9% of the global population) [1]. Angiotensin-I converting enzyme (EC 3.4.15.1, ACE), a main regulator in renin-angiotensin system, plays a pivotal role in catalyzing the conversion of angiotensin I to angiotensin II (a vasoconstrictor) and inactivating the bradykinin, a vasodilatory peptide, reducing blood pressure [2]. Thus, effective inhibition of ACE has been regarded a practical approach in reducing blood pressure. The food-derived ACE-inhibitory peptides are reported to be safer, compared with some synthetic ACE inhibitors, which may provoke several side effects [3].

In silico analysis, an effective approach to predict the release of bioactive peptides encrypted in protein sequences, can enlighten unstudied

proteins as precursors of bioactive peptides [4]. Compared with traditional experimental works, it is time-saving and more economical to investigate bioactive peptides through *in silico* analysis. Bioinformatic approaches, such as BLAST (alignment search tool for homology similarity analysis) [5], BIOPEP database [6] and quantitative structure-activity relationship (QSAR) [7] have been successfully applied to predict and design bioactive peptides from food-derived proteins, which help circumvent several challenges of classic approaches.

The meat industry generates a considerable amount of slaughter by-products. Connective tissue, as a major constituent of meat slaughter by-products, is abundant of collagen, which contains high amounts of Gly and Pro residues. Collagen peptides harboring these amino acids can display potential ACE inhibitory, antioxidant, antithrombotic, bone health promoting and dipeptidylpeptidase IV activities [3]. However, the evaluation of bovine collagen as a potential precursor of ACE inhibitory peptides using *in silico* approach is rarely reported. Furthermore, some previous *in silico* analysis studies failed to validate the exact activities of predicted bioactive peptides. Therefore, the aim of this study is to evaluate and predict the potential ACE inhibitory peptides encrypted in the bovine collagen sequences based on BLAST, BIOPEP and QSAR analyses.

II. MATERIALS AND METHODS

The representative protein sequences of collagen alpha-1(I) chain and collagen alpha-2(I) chain [Bos taurus] (NCBI Accession No: gi|77404252 and NCBI Accession No: gi|8039779) were selected from protein database of NCBI and used in our current study. The homology analysis was

performed using the protein sequences of collagen alpha-1(I) chain and collagen alpha-2(I) chain. As two or more sequences in the format of FASTA can be aligned, two collagen sequences were aligned with each other by aid of BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). A pairwise sequence alignment was generally displayed, while a series of instructive data could be generated [5].

The assessment of potential ACE inhibitory peptides from bovine collagen was carried out using BIOPEP database analysis (<http://www.uwm.edu.pl/biochemia/index.php/pl/biopep>) [8]. Firstly, the representative protein sequences of collagen alpha-1(I) chain and collagen alpha-2(I) chain were examined by “profiles of potential biological activity” tool; during this process, a number of potential collagen-derived ACE inhibitory peptide sequences were examined and compared with the reported 2609 peptide sequences with *in vitro/in vivo* ACE inhibitory capacity. In addition, the frequency of occurrence of bioactive fragments in the protein chain “A” was applied to characterize the potential activity of protein fragments according to the equation:

$$A=a/N$$

Where “a” is the number of fragments with given activity within the protein sequence and “N” is the number of amino acid residues within this protein chain [8].

Collagen sequences were subjected to *in silico* proteolysis for prediction of the theoretical peptide sequences cleaved by twenty seven different enzymes provided by the database. Additionally, a combination of enzymes (pepsin and trypsin, and pepsin, trypsin and chymotrypsin) was applied in order to mimic *in vitro* gastrointestinal digestive process. Afterwards, the theoretical peptides released by a variety of proteases were subjected to analysis by “search for active fragments”. Eventually, a list of potential peptides with ACE inhibitory activity was generated while exclusive ACE inhibitory peptides were chosen for further evaluation.

Two ACE inhibitory peptide databases consisting of 105 di-peptides and 140 tri-peptides collected from previously published works were constructed

to establish QSAR models [9]. The sequences of peptides were transformed into X-matrix by means of z-scales. Partial least squares (PLS) regression analysis between amino acid descriptors (predictors, X) and log-transformed IC₅₀ values (dependent, Y) was carried out using Simca 13 (Umetrics, Umeå, Sweden). The regression coefficients with confidence intervals were analyzed. All variables were auto-scaled to unit variance prior to the analyses. The number of significant PLS components was automatically selected based on RMSE. In an attempt to improve the predictive ability of the model, the U vs. T and Residual Variance vs. T² analysis were performed. The outliers, displaying much worse fit than others, were excluded. The lowest value of RMSE in the validation was chosen for further prediction of unknown ACE inhibitory activity. The optimal model was selected, where a reasonable balance between the models’ fit and predictive ability was achieved. The models were further validated initially by cross-validation during modelling. Eventually, the ACE inhibitory activities of collagen peptides produced by *in silico* hydrolysis were predicted by the optimal predictive models.

III. RESULTS AND DISCUSSION

The homology assessment data from the BLAST analysis of two collagen sequences are displayed in Table 1; the “identities” serves as the proportion of matched amino acids in the entire length of the aligned sequences; i.e. the larger the identities, the more homology; the “positives” stands for the values of matched amino acids and the substitutions “+” within the entire length; the “gaps” accounts for the values of “-” within the entire length [5]. According to the BLAST results, there is a substantial similarity of protein sequences between collagen alpha-1(I) and collagen alpha-2(I) with 65% sequence homology, 73% matched amino acids, 1% gap and 1024 bits out of 1678 within the total length (Table 1). The homologous proteins confer similar characteristics of potentially bioactive activity in protein fragments.

The occurrence frequency of ACE inhibitory peptides in collagen (parameter A) is displayed in Table 2. The values of parameter “A” for

collagen alpha-1(I) and collagen alpha-2(I) are 0.554 and 0.562, respectively. These values are much higher than the 0.225 for β -casein [8], which is in agreement with the fact that collagen and elastin present exceptionally large values of “A” of bioactivities [6].

Table 1 Summary of protein sequence alignments for bovine collagen alpha-1(I) and collagen alpha-2(I)

Alignment	Identities	Positives	Gaps	Scores
alpha-1(I) vs. alpha-2(I)	65%	73%	1%	1024 bits

The numbers of the theoretic ACE inhibitory peptides released by various proteases are summarized in Fig. 1. There were overall 782 and 775 potential ACE-inhibitory peptides for released from alpha-1(I) and alpha-2(I) collagen, respectively. Papain (100), chymotrypsin C (91), and ficain (89) were demonstrated to be the most effective enzymes in producing the most ACE inhibitory peptides for both collagen types; among them, a number of sequences (GP, PG, PR, AG, GA, GPA and LPG) were the most frequent peptides. These enzymes generated specifically numerous peptides with hydrophobic amino acids at the C-terminal positions such as Pro, Leu, Tyr, Ala, and Val. However, the gastrointestinal enzymes (pepsin, trypsin and chymotrypsin) failed to produce a large amount of ACE inhibitory peptides. The present BIOPEP analysis indicates that bovine collagen appears to be an excellent precursor of ACE inhibitors due to the higher possibility of the occurrence of ACE inhibitory pattern (Gly-Pro-Hyp).

Table 2 Occurrence frequency (A) of peptides with ACE inhibitory capacity with bovine collagen sequence

Name	Activity	A
collagen alpha-1(I)	ACE inhibitor	0.554
collagen alpha-2(I)	ACE inhibitor	0.562

By means of the established optimized model, the activity of 105 di-peptides and 140 tri-peptides prepared by *in silico* hydrolysis were

predicted in Table 3. The IC_{50} values from collagen-derived ACE inhibitory peptides range from 1.27 to 25792.30 μ M; the IC_{50} values of YK was the lowest (1.27 μ M), compared with the rest of the predicted di-peptides and tri-peptides. A number of peptides predicted in our study have been reported to be powerful ACE inhibitory peptides, with IC_{50} values close to our current predicted results.

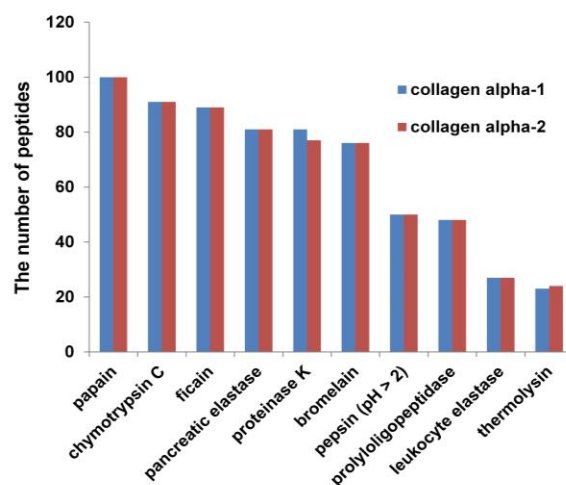


Figure 1. The predicted ACE inhibitory peptides released by various proteases (Top 10 of 27)

Table 3 The predicted ACE-inhibitory peptide sequences (Top 5)

Di-peptides	IC_{50} (μ M)	Tri-peptides	IC_{50} (μ M)
YK	1.27	LRP	7.69
AR	2.89	LRY	10.91
HK	4.52	GRP	16.48
NK	7.71	GKP	25.43
PQ	9.07	LTF	36.93

For example, VW had an IC_{50} value of 7.74 μ M, which is similar to the reported value 10.15 μ M, while the IC_{50} of AP (142.45 μ M) is akin to the calculated value (135.22 μ M) [10]. LRP (IC_{50} = 2.26) exhibited similar ACE inhibitory activity with actual values (IC_{50} = 1.00 μ M) [11]. These similarities demonstrate that our current QSAR models displays information and a satisfactory predictive ability concerning the predicted ACE

inhibitory peptides, which contributes to evaluating bovine collagen as a potential precursor of ACE-inhibitory peptides.

IV. CONCLUSION

Bovine collagen is a satisfactory precursor of ACE inhibitory peptides based on *in silico* analyses. BLAST analysis suggested that the amino acid sequences of collagen alpha-1(I) and collagen alpha-2(I) were homologous to each other. By aid of BIOPEP analysis, they were shown to be good precursors for producing ACE inhibitory peptides; *in silico* hydrolysis catalyzed by papain can theoretically release the most collagen-derived ACE inhibitory peptides. The ACE inhibitory activities of the resulting collagen peptides were predicted by QSAR models, indicating that collagen-derived peptides were potent ACE inhibitors with lower values of IC₅₀. Further experimental studies concerning hydrolysis of bovine collagen, characterization of the potent peptides and confirmation of the activity are in process.

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