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Production of Peptides Enhancing Calcium Solubility in the Presence of Phosphate Ions *In Vitro*

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In Vitro 상에서 인 이온 존재 하에서의 칼슘 용해도를 증대시키는 펩타이드의 생산

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Abstract

Gluten peptide was produced from corn gluten by enzymatic hydrolysis. This peptide had an ability to increase the solubility of calcium owing to protect calcium ions from forming precipitates of calcium phosphate in the presence of phosphate ions. The solubility of calcium was increased 5.2 times in the presence of 8.3 mg peptide produced by the treatment of papain. These peptides contained high acidic amino acids and fractionated by Delta pack column into fractions No. 1, No. 2 and No. 3. Among them the fraction No. 3 had the highest calcium binding capacity.

Key words : gluten peptide, calcium solubility.

INTRODUCTION

Enzymatic hydrolysis of food proteins is an ancient technology by which mankind has been able to improve the palatability and storage ability of the available protein resources. Most of general developments in enzymatic hydrolysis of food proteins are focused on soya protein and milk protein. Corn gluten was a by-product of corn starch industries and it was used as a feed, in particular in poultry feed blends where its content of xanthophyll was desirable. We focused on the utilization of corn gluten for the production of functional peptides such as casein phosphopeptide(CPP). Casein was a high quality protein but its cost was expensive. So we conducted this experiment to substitute corn gluten for casein. Corn gluten has been treated as a low grade protein because it has a little content of essential amino acids(lysine, tryptophan)[1]. Recently, several physiologically important functional peptides were developed from corn gluten[2~3].

Minerals are important micronutrient for human life. Calcium is more important micronutrient than anything else, because this mineral has essential metabolic functions and many people have calcium deficiency or iron deficiency[4]. Casein phosphopeptide was developed to improve the solubility and to increase the bioavailability of calcium in small intestine[5~7]. The peptides is characterized by their high content of phosphorylated serine-amino acid[8]. However CPP was very expensive because it was produced from milk casein. So we tried to produce economically the peptides that have same physiological functionality as CPP.

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In this work, we have been investigated the application of peptides produced from corn gluten to increase the solubility of calcium in the presence of phosphate ions.

MATERIALS AND METHODS

1. Materials

Corn gluten was obtained from Sam Yang Genex Co., Ltd. (Seoul, Korea). Papain was obtained from Amano(Nagaya, Japan). Acetonitrile and water of HPLC grade were obtained from Burdick & Jackson(MI, USA). Wako Calcium C was obtained from Wako Pure Chemical Industries Ltd(Osaka, Japan). DAPHCl(d-l-a-b-Diaminopropionic acid monohydrochloric acid) was obtained from Fluka(Switzerland). All other chemicals were analytical grade.

2. Determination of calcium solubilizing ability

Measurement of calcium solubilizing ability of peptides was determined by amount of soluble calcium in 20mM potassium phosphate buffer(pH 7.0) solution in the presence or absence of peptides[9]. One milliliter of deionized water(DW) or peptides solution was added to one milliliter of 10mM calcium chloride solution and mixed well. 2ml of 20mM potassium phosphate buffer(pH 7.0) was added and this reaction mixture was kept at 37℃ in a thermostatic bath. Small volume of the reaction mixture was filtered through a membrane with a pore size of 0.45㎛(Millipore, USA) for analysis of soluble calcium. Soluble calcium was determined by Wako Calcium C reagent or ion chromatography method(Dionex, USA).

3. Quantitative determination of soluble calcium

Soluble calcium was quantitatively determined by o-cresolphthalein complexon(OCPC) method[10]. 2.5ml of Wako buffer reagent was added to 25㎕ of sample solution and mixed well. 0.25ml of color reagent was added and vortexed for 20 seconds. After 5 minutes, the absorbance of solution was measured at 570nm(UVIKON 860, USA). Soluble calcium was also quantitatively determined by ion chromatography method. When soluble calcium was determined by ion chromatography method, a Dionex Ionpac-CS3 column(4mm×250mm) was used. The suppressor was CMMS(Cation micromembrane suppressor) and suppressor solution was 100mM TBAOH(Tetrabutylammonium hydroxide) solution. The eluent was a mixture of 48mM HCl and 16mM DAPHCl solution(1:1, v/v) and the flow rate was 1ml/min.

4. Fractionation of peptides

The peptides produced from corn gluten were fractionated by a BioCAD perfusion chromatography from Perseptive Biosystems(MA, USA). For the preparation of peptides, we used Delta Pak column(19mm×300mm, Millipore, USA). The eluent was a 0.1% TFA(Trifluoroacetic acid) in DW and 0.08% TFA in acetonitrile, and the flow rate was 5ml/min. The eluate was detected by using a UV detector at 214nm and was collected by fraction collector at an interval of one minute. These fractions were divided into three portions and concentrated by using of evaporator(Buchi, Switzerland) for removing TFA and acetonitrile at 85℃.

5. Determination of amino acid composition

Amino acid composition of peptides was determined by AccQ-Tag method using HPLC. Peptide solution was hydrolyzed in 6N HCl by heating at 110℃ for 24h. The content of tube was diluted with water in 50ml volumetric flask. 70㎕ of borate buffer was added to 10㎕ of sample solution and mixed with vortex. 20㎕ of AccQ-Fluoro reagent was added this solution and vortexed for 30 seconds and heated for 10 minutes at 55℃ in heating block. The eluent was AccQ-Tag buffer (pH 5.02), 60% Acetonitrile and Milli-Q water and the flow rate was 1.0 ml/min. We used AccQ-Tag column(3.9mm×150mm, Millipore, USA) and column oven temperature was 37℃. The eluate was detected by using a fluorescence detector.

6. Production of Peptides

The 15%(w/v) corn gluten solution was pre-

pared in water and adjusted to pH 6.0 with NaOH. Crude papain was added to corn gluten solution and the reaction mixture was incubated at 55℃ for 24hr. Peptides solution was harvested by centrifugation(6,000×g, 20min.) and concentrated by evaporation. The concentrated peptides solution was spray-dried. Small volume of the reaction mixture was taken for measuring calcium solubilizing ability at the desired intervals and treated in a boiling water bath for 15 minutes to stop the reaction. The sample solution was centrifuged at 8,000×g for 10 minutes for removing unhydrolyzed corn gluten particles.

RESULTS AND DISCUSSION

1. Production of peptides

For the production of peptides which enhanced calcium solubility in the presence of phosphate ions, we used a corn gluten as a raw material. We tested several kinds of proteases such as trypsin, pepsin, fungal protease, papain, and bromelain. Unfortunately, trypsin, pepsin, and fungal protease did not degrade gluten protein effectively (data was not shown). Plant protease such as bromelain and papain degraded gluten protein.

When 15% corn gluten was used with 1% papain at 55℃, gluten peptide was exponentially produced for 3 hrs. The total amount of peptides and production yield were 42.7g/l and 28.4% at 24 hrs, respectively(Fig. 1).

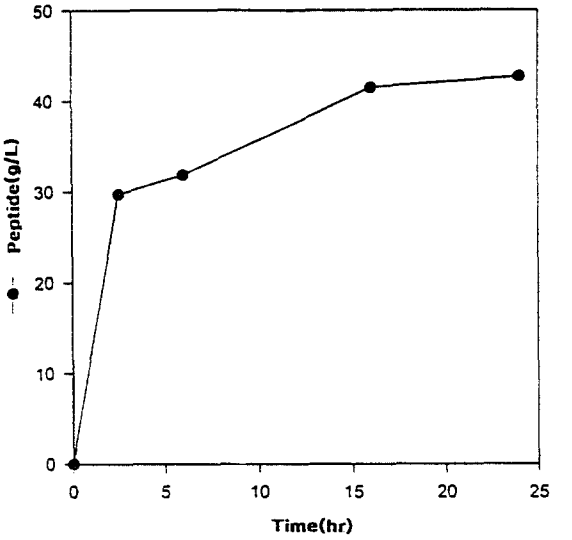

Fig. 1. Production of peptides from 15% corn gluten at 55℃ for 24 hrs.

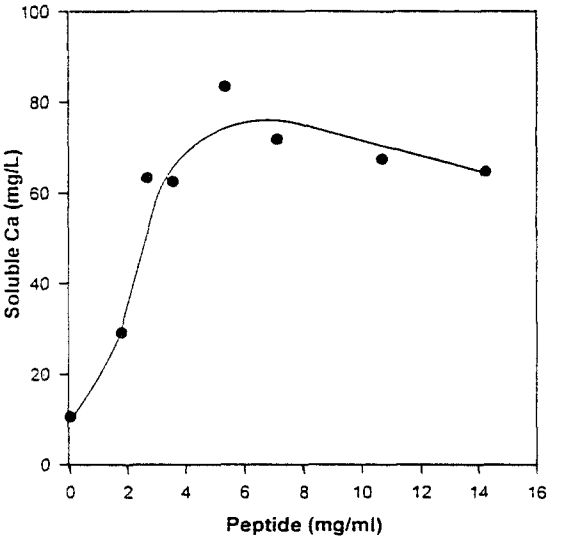

Fig. 2. Solubility of calcium in the presence of peptides.

2. Effect of Peptides on Calcium Solubility

As shown in Fig. 2, calcium solubility in the presence of phosphate ions was increased by gluten peptides. The amount of soluble calcium was 83.4mg/ml in the presence of 5.33mg/ml peptides and 15.2mg/ml in the absence of peptides. The solubility of calcium was increased 5.5 times in the presence of peptides. Calcium ions were formed precipitates with phosphate ions in the absence of peptides. However, the solubility of calcium ions was increased by formation of micelles in the presence of the peptides. Because corn gluten has high content of acidic amino acid, the peptides produced from corn gluten can form micelles with calcium ions. It is expected that other alkaline-earth-metal cations(Mg^{2+}, Ba^{2+}, Sr^{2+}) form soluble complexes with the peptides be-

cause these cations at low concentrations formed soluble complexes with bovine caseins[11~12]. We will tested the effect of gluten peptide enhancing solubility of calcium *in vivo*.

3. Effect of amount of papain

Proteolytic enzymes were derived from a wide range of sources such as bacteria, fungi, animals and plants. Corn gluten was degraded by plant proteases(papain, bromelain). The amount of peptides produced from corn gluten was increased in proportion to the amount of papain. But calcium solubilizing ability of peptides was not proportioned to the amount of papain(Fig. 3). 1% papain was reasonable to produce peptide because of high cost of papain. In high dosage of papain, many kinds of peptides and byproducts were produced and formed micelles with each other.

4. Peptides fractionation and calcium solubility capacity

Peptides were fractionated by a BioCAD perfusion chromatography and divided into 3 portions according to hydrophobicity(Fig. 4). We investigated calcium solubilizing capacity of each peptide fraction. As shown in Fig. 5, fraction No. 3

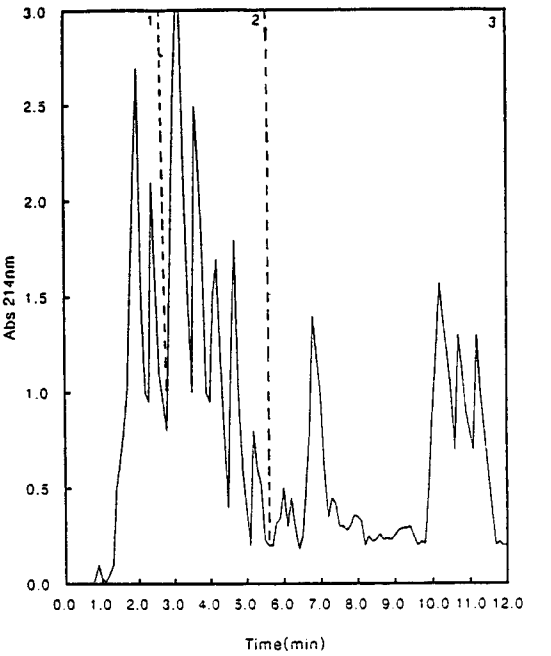

Fig. 4. Chromatogram of peptide according to hydrophobicity.

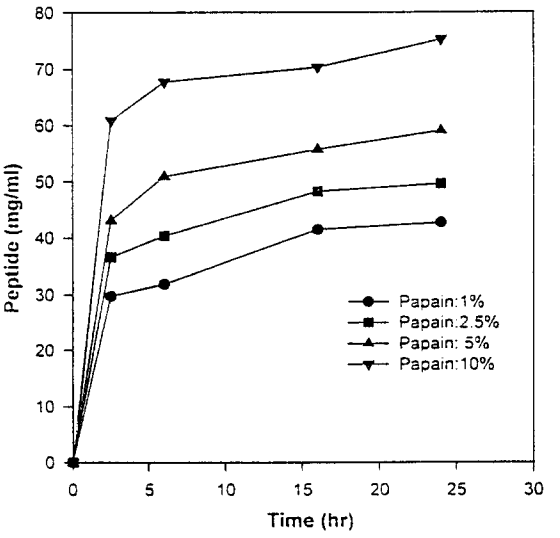

Fig. 3. Effect of papain concentration on the production of peptides.

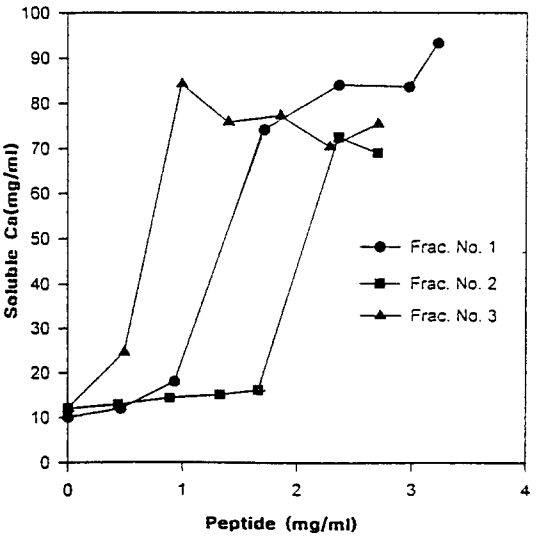

Fig. 5. Effect of peptide fraction on calcium solubility.

had a high calcium binding capacity. The relative order of binding capacities of peptide fraction to calcium ion is : fraction No. 3 > fraction No. 1 > fraction No. 2 > digested peptides. The digested peptides had lower binding capacity for calcium ions than that fractionated peptides. Fractionations of digested peptides might be attributed to remove the interaction between negative charged peptides and positive charged peptides or byproducts. Purification of peptides and elucidation of mechanism of interaction between calcium and the peptides are in progress in our laboratory. The difference of amino acid composition of fractionated peptides was investigated by HPLC method. As shown in table 1, fraction No. 1 had a high content of glutamic acid and aspartic acid compared to fraction No. 2 and No. 3. The content of acidic amino acids in gluten peptides, fraction No. 1, fraction No. 2 and fraction No. 3 is about 24.3%, 28.1%, 22.5% and 22.4%, respectively. We think that acidic amino acids are important factor to form micelles with calcium ions because there is no evidence of phosphoryl group in gluten peptides like CPP. However the sequence and the environment of acidic amino acids in peptides are more important factor because there was a little relationship between calcium binding capacity and acidic amino acids content. It seems that amino acid sequence of peptides and the environment of acidic amino acids in peptide are more important than amino acid composition of peptides.

CPP was able to promote the calcium absorption in chickens when injected into ligated loops of the small intestine[13]. We thought that peptides produced from corn gluten were able to in-

Table 1. Amino acid composition of gluten peptides

Name	Amino acid compositions(%)[a]			
	Whole peptide(%)	Frac. No. 1(%)[b]	Frac. No. 2(%)[c]	Frac. No. 3(%)[d]
ASP	6.13	6.61	5.24	5.1
SER	6.36	8.4	5.43	4.9
GLU	18.23	21.47	17.28	17.25
GLY	5.96	7.8	4.69	2.96
HIS	1.79	1.82	1.05	-
ARG	2.57	4.05	1.1	-
THR	3.69	4.61	3.07	1.26
ALA	12.32	16.95	11.9	8.66
PRO	10.18	6.95	12.3	17.02
TYR	3.27	2.33	4.73	2.95
VAL	4.70	4.97	4.97	4.90
MET	1.96	2.24	1.80	1.62
LYS	1.89	2.28	1.25	0.74
ILE	3.54	2.07	4.07	5.7
LEU	13.46	6.87	16.99	17.89
PHE	3.95	0.94	8.86	9.05

a : Values are mean values of triplicate.
b : Hydrophilic peptides
c : Semihydrophobic peptides
d : Hydrphobic peptides

crease solubility of calcium and to promote the absorption in small intestine. It is possible that these peptides can be used as functional peptides that promote calcium absorption in small intestine. We think that these peptides are effective to increase the bioavailability of calcium *in vivo*.

요 약

옥수수 글루텐을 식물성 효소인 파파인으로 가수분해하여 글루텐 펩타이드를 생산하였다. 생산된 글루텐 펩타이드류는 인이온 존재 하에서 인이온과 칼슘이온과의 침전형성을 방지하여 칼슘의 용해성을 증진시켰다. 칼슘이온의 용해도는 8.3mg의 펩타이드 존재시 대조구에 비해 5.2배 증대되었다. 산성 아미노산의 함량이 매우 높은 이들 펩타이드류를 Delta pak 칼럼을 이용하여 분획하였으며 이들 분획가운데 3번째 분획이 가장 높은 칼슘 용해성을 보였다.

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