Development And Bioproduction Of Protein Containing The Caseinophospho-peptide For Anti-osteoporosis

顏品澄、簡宏堅

E-mail: 364884@mail.dyu.edu.tw

ABSTRACT

Anti-ostepoprosis phosphorpeptide (CPP) is discovered from the casein protein of milk. Only β-casein has anti-osteoporosis activity in casein protein including αs1-casein, αs2-casein, β-casein, and κ-casein. B-casein was digested into 25 amino acid peptide by protease in the small intestine. A partial casein protein was precipitated with the calcium phosphate salts because the member is growing too big, caused the small intestine to be unable to absorb the casein protein with the calcium phosphate union macro-molecule. Casein protein was phosphorylated by casein kinase Ⅰ (CKⅠ) in mammary gland and then enters the small intestine to be digested into casein phosphopeptide. Only casein phosphopeptide was bound with calcium ions, and then absorbed by the small intestine. Therefore this laboratory use CKI to phosphorylate casein protein, and help casein phosphopeptide to bind with calcium ions and to be absorbed in the small intestine. Casein phosphopeptide R E L E E L N V P G E I V E S L S S S E E S I T R with four serine amino acid phosphorylated as indicated can bind with calcium ions to promote the absorption. In order to establish the high yield and the highly concentrated quantity producer type of CPP peptide, this laboratory uses the human tyrosine hydroxylase (HTH) to carry the casein phospho peptide, and also seeks HTH not to affect its activity when its five domains was replaced for the casein phosphorpeptide. As well as the N and C terminals of casein phosphopeptide are designed Phe to favor the future to be cut with pepsin for releasing the free casein phosphopeptide absorbed directly in the duodenum. Therefore we first carry on the design of primers for replacement and construction of casein phosphopeptide, and use overlap-PCR technique to construct the replacement of five domains for casein phosphopeptide, respectively, and then, insert in the pQE30 and unite five domains of casein phosphopeptide in one CPP-HTH. Gene expression system of pYLSC1-5S was used to reclone CPP-HTH and insert in the 5S-rDNA of Yarrowia lipolytica chromosome, called Y-CPP yeast. One the other hand we choose the CK1 gene to clone into internal secretion system of pYLEX1 and insert in the leucine biosynthetic gene of Y-CPP yeast, called Y-CPP-CKⅠ yeast, for bioproduction of casein phosphopeptide. The growth curve of Y-CPP and Y-CPP-CKI in growth medium were determined to be 16 h for the optimal growth with the spectrophotometer. The induction curve of Y-CPP and Y-CPP-CKⅠ growing in the inducing medium were determined to be 36 h for the massive production of CPP-HTH with the analysis of HPLC. The peptidyl protein CPP-HTH was concentrated by using the corn starch adsorption. The molecular weight of CPP-HTH is theoretically 54.6 kDa, however, the translation modification in the Y.lipolytica is to make its molecular weight to increase approximately 10 kDa. Therefore, the molecular weight of CPP-HTH is analyzed approximately 64.6 kDa with SDS-PAGE, but if CPP-HTH is phosphorylated by CKI to gain slightly high 64.6 kDa, if joins calcium ions consecutively to make even higher molecular weight.

Keywords : Yarrowia lipolytica

Table of Contents

REFERENCES

1. Choi, H.-I., Kim, H.-J., Park, J.-I., Shin,E.-H., Kim, D.-W. and Kim, S.-S. Design and efficient synthesis of novel ascorbyl conjugated peptide with high collagen biosynthesis stimulating effects. Bioorg. Med. Chem. Lett. 2009 ,19(7):2079 – 2082. 2. Claudia Gravaghi1, Elena Del Favero1, Laura Cantu'1, Elena Donetti2, Marzia Bedoni2, Amelia Fiorilli1, Guido Tettamanti1, Anita Ferraretto1. Casein phosphopeptide promotion of calcium uptake in HT-29 cells - relationship between biological activity and supramolecular structure,Article first published online: 29 AUG 2007DOI: 10.1111/j.1742-4658.2007.06015.x 3. Madzak C, Gaillardin C, Beckerich JM. Heterologous protein expression and secretion in the non-conventional yeast Yarrowia lipolytica: a review. J Biotechnol, 2004 ,109(1-2):63-81 4. Meisel, H.1,* and FitzGerald, R. J2. Biofunctional Peptides from Milk Proteins: Mineral Binding andCytomodulatory Effects, Current Pharmaceutical Design, 2003, 9, 1289-1295 5. Sambrook J, and Russell D.W. Molecular cloning : a laboratory manual, third edition.Cold spring harbor laboratory press, 2001 . NewYork. 6. Goodwill KE, Sabatier C, Stevens RC. CrystalStructure of Tyrosine Hydroxylase with Bound Cofactor Analogue and Ironat 2.3 A* Resolution: Self-Hydroxylation of Phe300 and the terin-BindingSite. Biochemistry, 1998 ,37(39):13437-13445. 7. Kenneth E. Goodwill, Christelle Sabatier, and Raymond C. Stevens. Crystal Structure of Tyrosine Hydroxylase with Bound Cofactor Analogue and Ironat 2.3 A Resolution: Self-Hydroxylation of Phe300 and the Pterin-Binding Site.Biochemistry. 1998 ,37(39):13437-13445. 8. Manley CH,and Ahmedi S. The development of process flavors. Trends Food Sci Technol. 1995 , 6(2):46-51. 9. Katayama, K., Armendariz-Borunda, J., Raghow, R., Kang, A.H.

and Seyer, J.M. A pentapeptide from type I procollagen promotes extracellular matrix production. J. Biol. Chem. 1993 ,268(14):9941 – 9944 . 10. Katayama, K., Seyer, J.M., Raghow, R. and Kang, A.H. Regulation of extracellular matrix production by chemically synthesized subfragments of type I collagen carboxy propeptide. Biochemistry. 1991 ,30(29):7097 – 7104 . 11. Nishimura A., Morita M., Nishimura Y.,and Sugino Y. A rapid and hifhly efficient method for preparation of competent Escherichia coli cells.Nucleic acid research. 1990 , 18:61-69.