利用嗜甲醇酵母菌產製重組酪蛋白磷酸胜?忖T元體及其促鈣吸收功能分析

王冠傑、陳小玲

E-mail: 387170@mail.dyu.edu.tw

摘 要

酪蛋白磷酸胜?（casein phosphopeptides，簡稱為CPPs）是酪蛋白的胜?片段，能與鈣離子鍵結增加其溶解度，避免鈣離子與磷酸鹽結合為難溶於水的磷酸氫鈣，促進鈣離子於腸道的吸收效率。這種能結合鈣離子的功能是由於CPPs具有三個磷酸化絲胺酸及兩個麩胺酸的特殊的胺基酸序列，稱之為“ acidic motif ”。CPPs為αS1、 αS2及β-酪蛋白經胰蛋白?和胰凝乳蛋白?於動物體外降解的產物，市面上大部分的CPPs為混雜多種不同胺基酸序列的產品，這類產品較難應用至醫藥產品。本試驗中構築β-酪蛋白磷酸胜?三元體基因於pGAPZαC表現載體，並轉型於Pichia pastoris GS115酵母菌株，利用攪拌式發酵槽大量發酵生產，並以鎳離子親和性管柱進行β-酪蛋白磷酸胜?三元體之純化。最後證實經純化之重組胜?在Caco-2細胞株試驗模式，具有促進鈣離子吸收之生物活性。

關鍵詞 : 酪蛋白磷酸胜?、嗜甲醇酵母、鈣離子吸收

目錄

參考文獻

1.白禮源、丘思穎、林怡年、林志聰、蔡秀欣、吳志成、吳柏林、陳永學、黃忠英、劉伯宏 編譯。1991。甘龍醫用生理學。pp566-568。藝軒圖書出版社。台北。 2.八幡義人。2011。血液好順暢!圖解全循環。pp150-154。書泉出版社。台北。 3.鄭秀卿。2005。以動物模式建立營養素或藥品影響骨骼發育的評估方法:pp10-11。中原大學大學碩士畢業論文。桃園。 4.林以勤、潘文涵。2009。台灣地區19歲以上成人骨密度狀況初探:2007-2008之狀況。 5.吳幸娟、潘文涵、葉乃華、張新儀、洪淑怡。2009。台灣成年與老年人營養素及食物攝

取來源之變遷趨勢:由NASHIT 1993-1996至2005-2008。 6.衛生署。2012。國人膳食營養素參考攝取量及其說明。行政院衛生署。台北。 7.尤新 編著。2001。機能性發酵製品。pp229-239。藝軒圖書出版社。台北。 8.林自威。2004。S. cerevisiae與P. pastoris高密度發酵生產葡萄糖氧化?及其酵素特性比較:pp115-130。國立台灣科技大學碩士畢業論文。台北。 9.Abrams SA & Stuff JE (1994) Calcium metabolism in girls: current dietary intakes lead to low rates of calcium absorption and retention during puberty. The American journal of clinical nutrition 60: 739-743. 10.Alderman BW, Weiss NS, Daling JR, Ure CL & Ballard JH (1986) Reproductive history and postmenopausal risk of hip and forearm fracture. American journal of epidemiology 124: 262-267. 11.Anderson JJ (2001) Calcium requirements during adolescence to maximize bone health. Journal of the American college of nutrition 20: 186S-191S. 12.Bailey DA, Martin AD, McKay HA, Whiting S & Mirwald R (2000) Calcium accretion in girls and boys during puberty: a longitudinal analysis. Journal of bone and mineral research 15: 2245-2250. 13.Berrocal R, Chanton S, Juillerat MA, Pavillard B, Scherz J-C & Jost R (1989) Tryptic phosphopeptides from whole casein. II. Physicochemical properties related to the solubilization of calcium. The journal of dairy research 56: 335-341. 14.Bramanti E, Sortino C, Onor M, Beni F & Raspi G (2003) Separation and determination of denatured alpha(s1)-, alpha(s2)-, beta- and kappa-caseins by hydrophobic interaction chromatography in cows', ewes' and goats' milk, milk mixtures and cheeses. Journal of chromatography A 994: 59-74. 15.Choi J, Horne D & Lucey J (2011) Determination of molecular weight of a purified fraction of colloidal calcium phosphate derived from the casein micelles of bovine milk. Journal of dairy science 94: 3250-3261. 16.Dalgleish DG, Spagnuolo PA & Douglas Goff H (2004) A possible structure of the casein micelle based on high-resolution field-emission scanning electron microscopy. International dairy journal 14: 1025-1031. 17.Farrell Jr H, Malin E, Brown E & Qi P (2006) Casein micelle structure: What can be learned from milk synthesis and structural biology? Current opinion in colloid & interface science 11: 135-147. 18.Farrell HM Jr, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, Hicks CL, Hollar CM, Ng-Kwai-Hang KF & Swaisgood HE (2004) Nomenclature of the proteins of cows ’ milk—sixth revision. Journal of dairy science 87: 1641-1674. 19.Ferraretto A, Gravaghi C, Fiorilli A & Tettamanti G (2003) Casein-derived bioactive phosphopeptides: role of phosphorylation and primary structure in promoting calcium uptake by HT-29 tumor cells. Federation of european biochemical societies letters 551: 92-98. 20.Ferraretto A, Signorile A, Gravaghi C, Fiorilli A & Tettamanti G (2001) Casein phosphopeptides influence calcium uptake by cultured human intestinal HT-29 tumor cells. The journal of nutrition 131: 1655-1661. 21.Goodrick J, Xu M, Finnegan R, Schilling B, Schiavi S, Hoppe H & Wan N (2001) High?level expression and stabilization of recombinant human chitinase produced in a continuous constitutive Pichia pastoris expression system. Biotechnology and bioengineering 74: 492-497. 22.Greenspan SL, Maitland LA, Myers ER, Krasnow MB & Kido TH (1994) Femoral bone loss progresses with age: a longitudinal study in women over age 65. Journal of bone and mineral research 9: 1959-1965. 23.Hannan MT, Felson DT & Anderson JJ (1992) Bone mineral density in elderly men and women: results from the Framingham osteoporosis study. Journal of bone and mineral research 7: 547-553. 24.Hoenderop JG, Nilius B & Bindels RJ (2005) Calcium absorption across epithelia. Physiological reviews 85: 373-422. 25.Hoenderop JG, Vennekens R, Muller D, Prenen J, Droogmans G, Bindels RJ & Nilius B (2001) Function and expression of the epithelial Ca2+ channel family: comparison of mammalian ECaC1 and 2. The journal of physiology 537: 747-761. 26.Horne DS (1998) Casein interactions: casting light on the black boxes, the structure in dairy products. International dairy journal 8: 171-177. 27.Horne DS (2006) Casein micelle structure: models and muddles. Current opinion in colloid & interface science 11: 148-153. 28.Hunt CD & Johnson LK (2007) Calcium requirements: new estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. The American journal of clinical nutrition 86: 1054-1063. 29.Ilich JZ & Kerstetter JE (2000) Nutrition in bone health revisited: a story beyond calcium. Journal of the American college of nutrition 19: 715-737. 30.Invitrogen (2008) pGAPZ A, B, and C pGAPZ α A, B, and C Pichia expression vectors for constitutive expression and purification of recombinant proteins. 31.Jandal J (1996) Comparative aspects of goat and sheep milk. Small ruminant research 22: 177-185. 32.Jiang Y, Li Q, Yan H & Geng L (2004) Expression and bioactivity analysis of recombinant beta-CPP dimer. Journal of dairy science 87: 3198-3208. 33.Kalkwarf H (1999) Hormonal and Dietary Regulation of Changes in Bone Density During Lactation and After Weaning in Women. Journal of mammary gland biology and neoplasia 4: 319-329. 34.Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J & Ho M (1997) The effect of calcium supplementation on bone density during lactation and after weaning. The new england journal of medicine 337: 523-528. 35.Kellett GL (2011) Alternative perspective on intestinal calcium absorption: proposed complementary actions of Ca(v)1.3 and TRPV6. Nutrition reviewsutr 69: 347-370. 36.Kenny AM & Prestwood KM (2000) Osteoporosis: pathogenesis, diagnosis, and treatment in older adults. Rheumatic disease clinics of north america 26: 569-591. 37.Khanal RC & Nemere I (2008) Regulation of intestinal calcium transport. Annual review of nutrition 28: 179-196. 38.Kreiger N, Kelsey JL, Holford TR & O'Connor T (1982) An epidemiologic study of hip fracture in postmenopausal women. American journal of epidemiology 116: 141-148. 39.Leitch I & Aitken FC (1959) The estimation of calcium requirement: a re-examination. Nutrition abstracts and reviews. Series A: Human and experimental 29: 393-411. 40.Li Z, Xiong F, Lin Q, d'Anjou M, Daugulis AJ, Yang DS & Hew CL (2001) Low-temperature increases the yield of biologically active herring antifreeze protein in Pichia pastoris. Protein expression and purification 21: 438-445 41.Looker AC, Melton LJ, Harris TB, Borrud LG & Shepherd JA (2010) Prevalence and trends in low femur bone density among older US adults: NHANES 2005 – 2006 compared with NHANES III. Journal of bone and mineral research 25: 64-71. 42.Mahmood A & Usman S (2010) A comparative study on the physicochemical parameters of milk samples collected from buffalo, cow, goat and sheep of Gujrat, Pakistan. Pakistan journal of nutrition 9: 1192-1197. 43.Meisel H & Frister H (1988) Chemical characterization of a caseinophosphopeptide isolated from in vivo digests of a casein diet. Biological chemistry hoppe-seyler 369: 1275-1280. 44.Meisel H & Olieman C (1998) Estimation of calcium-binding constants of casein phosphopeptides by capillary zone electrophoresis. Analytica chimica acta 372: 291-297. 45.Meisel H, Bernard H, Fairweather-Tait S, FitzGerald RJ, Hartmann R, Lane CN, McDonagh D, Teucher B & Wal JM. (2003) Detection of caseinophosphopeptides in the distal ileostomy fluid of

human subjects. British journal of nutrition 89: 351-358. 46.Mellander O (1950) The physiological importance of the casein phosphopeptide calcium salts. 2. Peroral calcium dosage of infants. Some aspects of the pathogenesis of rickets. Acta societatis botanicorum poloniae 55: 247-255. 47.Moezizadeh M & Moayedi S (2009) Anticariogenic Effect of Amorphous Calcium Phosphate Stabilized by Casein Phosphopeptid: A Review. Article. research journal of biological sciences 4 (1): 132-136. 48.Morgan EL, Mace OJ, Helliwell PA, Affleck J & Kellett GL (2003) A role for Ca(v)1.3 in rat intestinal calcium absorption. Biochemical and biophysical research communications 312: 487-493. 49.Morgan EL, Mace OJ, Affleck J & Kellett GL (2007) Apical GLUT2 and Cav1.3: regulation of rat intestinal glucose and calcium absorption. The Journal of physiology 580: 593-604. 50.Morr CV (1967) Effect of oxalate and urea upon ultracentrifugation properties of raw and heated skimmilk casein micelles. Journal of dairy science 50: 1744-1751. 51.Naito H, Kawakami A & Imamura T (1972) In vivo formation of phosphopeptide with calcium-binding property in the small intestinal tract of the rat fed on casein. Agricultural and biological chemistry 36: 409-415. 52.Nakkrasae LI, Thongon N, Thongbunchoo J, Krishnamra N & Charoenphandhu N (2010) Transepithelial calcium transport in prolactin-exposed intestine-like Caco-2 monolayer after combinatorial knockdown of TRPV5, TRPV6 and Cav1. 3. The journal of physiological sciences 60: 9-17. 53.Nordin BE, WIshart JM, Clifton PM, McArthur R, Scopacasa F, Need AG, Morris HA, O'Loughlin PD & Horowitz M (2004) A longitudinal study of bone?related biochemical changes at the menopause. Clinical endocrinology 61: 123-130. 54.Orwoll ES, Oviatt SK, McClung MR, Deftos LJ & Sexton G (1990) The rate of bone mineral loss in normal men and the effects of calcium and cholecalciferol supplementation. Annals of internal medicine 112: 29-34. 55.Peng JB (2011) TRPV5 and TRPV6 in transcellular Ca2+ transport: regulation, gene duplication, and polymorphisms in African populations. Advances in experimental medicine and biology 704: 239-275. 56.Peng JB, Brown EM & Hediger MA (2003) Apical entry channels in calcium-transporting epithelia. Physiology 18: 158-163. 57.Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM & Hediger MA (1999) Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. Journal of biological chemistry 274: 22739-22746. 58.Peng JB, Chen XZ, Berger UV, et al. (2000) Human calcium transport protein CaT1. Biochemical and biophysical research communications 278: 326-332. 59.Perego S, Cosentino S, Fiorilli A, Tettamanti G & Ferraretto A (2012) Casein phosphopeptides modulate proliferation and apoptosis in HT-29 cell line through their interaction with voltage-operated L-type calcium channels. The journal of nutritional biochemistry 23: 808-816. 60.Perego S, Zabeo A, Marasco E, Giussani P, Fiorilli A, Tettamanti G & Ferraretto A (2013) Casein phosphopeptides modulate calcium uptake and apoptosis in Caco2 cells through their interaction with the TRPV6 calcium channel. Journal of functional foods. 61.Phadungath C (2005) Casein micelle structure: a concise review. Songklanakarin journal of science and technology 27: 201-212. 62.Poto?nik K, Gantner V, Kuterovac K & Cividini A (2011) Mare ' s milk: composition and protein fraction in comparison with different milk species. Mljekarstvo 61: 107. 63.Raman L, Rajalakshmi K, Krishnamachari K & Sastry JG (1978) Effect of calcium supplementation to undernourished mothers during pregnancy on the bone density of the bone density of the neonates. The American journal of clinical nutrition 31: 466-469. 64.Reynolds EC (1997) Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. Journal of dental research 76: 1587-1595. 65.Rosen CJ, Bouillon R, Compston JE & Rosen V (2013) Primer on the metabolic bone diseases and disorders of mineral metabolism. Wiley. 66.Ross AC, Manson JE, Abrams SA, et al. (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. Journal of clinical endocrinology & metabolism 96: 53-58. 67.Sato R, Noguchi T & Naito H (1986) Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. Journal of nutritional science and vitaminology 32: 67-76. 68.Sato R, Shindo M, Gunshin H, Noguchi T & Naito H (1991) Characterization of phosphopeptide derived from bovine β-casein: an inhibitor to intra-intestinal precipitation of calcium phosphate. Biochimica et biophysica acta (BBA)-protein structure and molecular enzymology 1077: 413-415. 69.Schmidt D (1982) Association of caseins and casein micelle structure. Developments in dairy chemistry 1: 61-86. 70.Seeley RR, Stephens TD & Tate P (2007) Essentials of anatomy and physiology. McGraw-Hill Higher Education. 71.Sharma S, Singh R & Rana S (2011) Bioactive Peptides: A Review. International journal 15. 72.Silva SV & Malcata FX (2005) Caseins as source of bioactive peptides. International dairy journal 15: 1-15. 73.Specker BL, Vieira NE, O'Brien KO, Ho ML, Heubi JE, Abrams SA & Yergey AL (1994) Calcium kinetics in lactating women with low and high calcium intakes. The American journal of clinical nutrition 59: 593-599. 74.Vatanparast H, Bailey DA, Baxter-Jones AD & Whiting SJ (2010) Calcium requirements for bone growth in Canadian boys and girls during adolescence. British journal of nutrition 103: 575. 75.Walstra P (1999) Casein sub-micelles: do they exist? International dairy journal 9: 189-192. 76.Wasnich R, Yano K & Vogel J (1983) Postmenopausal bone loss at multiple skeletal sites: relationship to estrogen use. Journal of chronic diseases 36: 781-790. 77.Werten MW, van den Bosch TJ, Wind RD, Mooibroek H & de Wolf FA (1999) High?yield secretion of recombinant gelatins by Pichia pastoris. Yeast 15: 1087-1096.