Studies on the production of inositol from phytic acid using immobilized phytase.

蔣正華、曾耀銘

E-mail: 8515653@mail.dyu.edu.tw

ABSTRACT

由 Aspergillus ficuum 所純化之植酸?（E.C.3.1.3.8）能將植酸 水解為肌醇。本實驗改變以往的判別法，利用高效液相層析儀（HPLC）來 定量此催化反應，研究以植酸為基質及肌醇生成速率為活性的各項性質， 得到酵素之最適溫度為 50 ℃ 左右，最適 pH 則在 5 附近，在此條件下 最大活性為 95.928 sec-1，與親和性有關的 Km 為 2.337×10-2 M，而 肌醇的產率約為 79.32 %。選擇食用明膠為擔體，欲將 A. ficuum 植酸 ?固定化，於系統中改變數種不同因素，測得最佳固定化條件，即利用 2.5 %戊二醛水溶液為交聯劑與偶合劑，與未成形之明膠於室溫下反應 90 分鐘，以增加其組織堅硬度，再將此明膠顆粒加入酵素溶液中，在 4 ℃ 下連接 12 小時，最後可得到每克擔體上有 0.068 毫克的酵素乾重， 此條件之最佳固定化率為 51 %。取此最佳交聯擔體進行固定化酵素反應 的測試，於相同的定性、定量方法下，發現固定化後的最適 pH 與未固定化的比較並無明顯改變，但卻提高其耐高溫的能力，最適溫度上升約 10 ℃。在動力學常數方面，其Km 增加為 3.276 ×10-2 M，在個別最適條件 下，固定化後之活性為游離酵素的 34.58 %，而肌醇的產率則減少為 23.06 %。

Keywords : phytase ; HPLC ; phytic acid ; inositol ; immobilization

Table of Contents

REFERENCES

1.王三郎.1991.生物工學入門.藝軒圖書公司 2.陳國誠.1989.微生物酵素工程學. 藝軒圖書公司 3.陳水田.1989.酵素催化合成生理活性物質. 台灣大學生化科學研究所博士論文 4.林佳慧.1996.真菌植酸?在食品工業之應用.食品工業月刊.,20-24. 5.Anderson, R.J.1915.Concerning phytin in wheat bran. J. Biol. Chem.,20,493-500. 6.Bitar ,K. and Reinhold, J.G.1972.Phytase and alkaline phosphatase activites in intestinal mucosae of rat, chicken, and man. Biochim. Biophys. Acta.,268,442-452. 7.Camilli, P.D., Emr, S.D., McPherson, P.S. and Novick, P.1996. Phosphoinositides as regulators in membrane traffic. Sci.,271,1533-1539. 8.Cerklewski, F.L.1992.Phytic acid plus supplemental calcium, but not phytic acid alone, decreases fluoride bioavailability in the rat. J.Nutr. Biochem.,3,87-90. 9.Churella, H.R. and Vivian, V.M. 1989. Effect of phytic acid level in soy protein based infant formulas on mineral availability in the rat. J.Agric. Food Chem.,37,1352-1357. 10.Davidson, L., Galan, P., Kastenmayer, P., Cherouvrier, F., Juillerat, M.A., Hercberg, S. and Hurrell, R. F.1994. Iron bioavailability studied in infants : The influence of phytic acid ane ascorbic acid in infant formulas based on soy isolate. Pediatr. Res.,36(6),816-822. 11.Draper, H.H. and Bird, R.P. 1984. Antioxidants and cancer. J. Agric. Food Chem.,32,433-435. 12.Eagle, H., Oyama, V.I., Levy, M. and Freeman, A.E. 1987. myo-inositol as an essential growth factor for normal and malignant human cells in tissue culture. J. Biol. Chem., 266, 191-205. 13.Eisenberg, F.1967. D-myoinositol l-phosphate as pro-duct of cyclization of glucose 6-phosphate and substrate for a specific phosphatase in rate testic. J. Biol. Chem.,242(7),1375-1382. 14.Elliott, S.,Chang, C.W.,Schweingruber. M.e., Schaller, J., Rickli, E.E.and Carbon ,J. 1986. Isolation and characterization of the structural gene for decreted acid phosphatase from Schizosaccharomyces pombe. J.Biol. Chem., 261(6),2936-2941. 15.Ellis, R.and Morris ,E. R.1986. Appropriate resin selection for rapid phytate analysis by ion -exchabge chromatograph. Cereal.,63(1),58-59. 16.Empson, K.L.,Labuza,T.P.and Graf,E.1991.Phytic acid as a food antioxidant.J.Food Sci.,56(2),560-563. 17.Farkvam,D.O.,Nelson,T.S.,Kirby,L.K.,Johnson,Z.B.and Stamps, A.T.1989. Calcium and phytic acid in chick nutrition.Nutr. Rep. Int.,40(1),33-42. 18.Fordyce, E.J..,Forbes, R.M.,Robbins,K.R.and Erdman, J.W.1987.Phytate × calcium/zinc molar ratios : Are they predictive of zinc bioavailability. J.Food Sci.,52(2),440-444. 19.Freinkel,N.,Younsi,C.E. and Dawson, M.C.1975.Interrelations between the phospholipids of rat pancreatic islets during glucose stimulation. Eur. J. Biochem.,59,245-252. 20.Gibbins, L.N.and Norris, F.W.1963.Phytase and acid phosphatase in the dwarf bean, phadeolus vulgaris. Biochem.J.,86,67-71. 21.Gibson,D.M.and Ullah, A.H.J.1988. Purification and characterization of phytase from cltyledons of germinating soybean seeds. Archives Biochem. Biophys.,260(2),503-513. 22.Graf, E.1983. Applications of phytic acid. J. Am. Oil Chem.Soc.,60(11),1861-1867. 23.Graf, E. and Eaton, J.W.1993. Suppression of colonic cancer by dietary phytic acid. Nutr. Cancer.,19,11-19. 24.Greene, D.A.and Lattimer, S.A.1982. Sodium and energy-dependent uptake of myo-inositol by rabbit peripheral nerve. J. Clin.Invest.,70,1009-1018. 25.Greene,D.A.,De jesus,P.V.and Winegrad,A.I.1975 Effect of insulin and dietary myoninositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes.J.Clin.Invest.,55,1326-1336. 26.Hallman,M.,Slivka,S., Wozniak,P. and sills,J.1986.perinatal development of myoninositol uptake into lungcells:Surfactant phosphatidylglycerol and phosphatidylinositol synthesis in the rabbit.Pediatr.Res.,20(2),179-185. 27.Harland, B.F.and Harland,J.1980.Fermentative reduction of phytate in rye white,and whole wheat breads.Cereal Chem.,57(3),226-229. 28.Hegsted,D.M.,Gallagher,A.and Hanford,H.1974 Inositol requirment of the gerbil.J.Nutr.,104,588-592. 29.Irving,G.C.J.and Cosgrove,D.J.1974.Inoistol phosphate phosphatases of microbiological origin.Some properties of the partially purified phosphatases of Aspergillus ficuum NRRL3135.Aust. J. Biol. Sci., 27, 361-368. 30.Karasawa,K.1972.The effect of carbohydrate and inositol on the growth of rats.Jph.J.Nutr.,30,3-11. 31.Laboure,A.-M.,Gagnon,J. and Lescure,A.-M.1993.Purification and characterization of a phytase (myo-inositol-hexakisphosphate phosphohydralase)accumulated in maize (Zeamays) seedlings during germination. Biochem.J.,295,413-419. 32.Lim,P.E.and Tate,M.E.1971.The phytases.I.Lysolecithin-activited phytase from wheat bran.Biochim.Biophys.Acta.,250,155-164. 33.Lim,P.E.and Tate,M.E.1973.The phytases.II.Properties of phytase fractions F1 and F2 from wheat bran and the myo-inositol phosphates produced by fraction F2.Biochim.Biophys.Acta.,302,316-328 34.Lonnerdal,B.,Sandberg,A.-S.,Sandstrom,B. and Kunz,C.1989.Inhibitory effect of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rates. J.Nutr.,119,211-214. 35.Maga,J.A.1982.Phytate : Its chemistry,occurrence,food interations,nutritional significance,and method of analysis.J.Agric Food Chem.,30(1),1-9. 36.Maiti,I.B.and Biswas,B.B.1973.Further characterization of phytase from Phaseolus aureus.Phytochem.,18,316-318. 37.Mandal,N.C..,Burman,S.and Biswas,B.B.1971.Isolation,purification and characterization of phytase from germinating mung beans.Phytochem.,11,495-502. 38.Matthaus,B., Losing,R. and Fiebig ,H.J.1995.Determination of phytic acid and its degradation products in extracts of rape seeds and rapeseed meal.J.High Resol.Chromatogr.,18,267-268. 39.McCance,R.A.and Widdowson,E.M.1935.Phytin in human nutrition.Biochem.J.,29,2694-2699. 40.McCance,E.V.and Hart,E.B.1908.On the occurrence of a phytin-splitting enzyme in animal tissues.J.Biol.Chem.,4,497-500. 41.Mellanby,E.1944.Phytic acid and phytase in cereals.Nature.,154,394-395. 42.Mrsa,V.,Barbaric,S.,Ries,B.and Mildner,P.1984.Phytase.FEBS Letters.,218(1),172-177. 43.Nagai,Y.and Funahashi,S.1962.Phytase from wheat bran.Agr.Biol.Chem.,26(2),794-803. 44.Nair,V.C.and Duvnjak,Z.1990.Reduction of phytic acid content in canola meal by Aspergillus ficuum in solid state fermentation process.Appl.Microbiol.Biotechnol.,34,183-188. 45.Nelson,T.S.1967.The utilization of phytate phosphorus by poultry a review.Poult.Sci.,46,862-871. 46.Nelson,T.S.,Shieh,T.R.,Wodzinski,R.J.and Ware,J.H.1968.The Availability of phytate phospharrus in soybean meal before and after treatment with a mold phytase.Poult.Sci.,47,1842-1848. 47.Nolan,K.B.and Duffin,P.A.1987.Effect of phytate on mineral bioavailability.In vitro studies on Mg2+,Ca2+,Fe3+,Cu2+ and Zn2+(also Cd2+) Solubilities in the presence of phytate.J.Sci.Food Agric.,40,79-85. 48.Nwokolo,E.N.and Bragg,D.B.1977.Influence of phytic acid and crude fiber on the availability of minerals from four protein supplements in growing chicks. Can.J.Anim.Sci.,57,475-477. 49.Patwardhan,V.N.1931.The occurrence of a phytinsplitting enzyme in the intestines of albino rats.Biochem.J.,31,560-564. 50.Peer.F.G.1953.The phytase of wheat.Biochem.J.,53,102-110.

51.Phillippy,B.Q.,White,K.D.,Johnston,M.R.,Tao,S.H.and Fox,M.R.S.1987.Preparation of inositol phosphates from sodium phytate by enzumatic and nonenzymatic hydrolysis.Anal.Biochem.,162,115-121. 52.Ranhotra,G.S.1973.Factors affecting hydrolysis during breadmaking of phytic acid in wheat protein concentrate.Cereal Chem.,50,353-357. 53.Ranhotra,G.S.1972.Hydrolysis during breadmaking of phytic acid in wheat protein concentrate.Cereal Chem.,50,353-357. 54.Ranhotra,G.S.,Loewe,R.J.and Puyat,L.V.1972.Phytic acid in soy and its hydrolysis during breadmaking.J.Food Sci.,39,1023-1025. 55.Rapoport,S.1940.Phytic acid in avian erythrocytes.J.Biol.Chem.,135,403-406. 56.Rapoport,S.,Leva,E.and Guest,G.M.1941.Phytase in plasma and erythrocytes of various species of vertebrates.J.Biol.Chem.,139,621-632. 57.Reinhold,J.G.,Faradji,B.and Abadi,P.1976.Decreased absorption of calcium,magnesium,zinc and phosphorus consumption as wheat bread.J.Nutr.,106,493-503. 58.Sandberg,A.-S.and Andersson,H.1988.The effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans.J.Nutr.,118,469-473. 59.Sartirana,M.L.and Bianchetti,R.1967.The effect of phosphate on the development of phytase in the wheat embryo.Physiol.Plantarun.,20,1066-1075. 60.Serraino,M.R.and Thompson,L.U.1984.Removal of phytic acid and protein - phytic acid interactions in rapeseed.J.Agric.Food Chem.,32,38-40. 61.Shieh,T.R.and Ware,J.H.1968.Survey of microorganisms for the production of extracellular phytase.Appl.Microbiol.,16(9),1348-1351. 62.Steveninck,R.F.M.,Babare,A.,Fernando,D.R.and Steveninck,M.E.1994.The binding of zinc,but not cadimium,by phytic acid in roots of crop plants.Plant and Soil.,167,157-164. 63.Tangkongchitr,U.,Seib,P.A.and Hoseney,R.C.1981.Phytic acid.I.Determination of three forms of phosphorus in flour,dough,and bread.Cereal Chem.,58(3),226-228. 64.Tangkongchitr,U.,Seib,P.A.and Hoseney,R.C.1981.Phytic acid.II.Its fate during breadmaking.Cereal Chem.,58(3),229-234. 65.Tangkongchitr,U.,Seib,P.A.and Hoseney,R.C.1982.Phytic acid.III.Two barriers to the loss of phytate during breadmaking.Cereal Chem.,58(3),216-221. 66.Turecki,T.,Ewan,R.C.and Stahr,H.M.1994.Effect of phytic acid and calcium on the intestinal absorption of cadmium in vitro.Bull.Environ.Contam.Toxicol.,53,464-470. 67.Ullah,A.H.J.1988.Aspergillus ficuum phytase : Partial primary structure,substrate selectivity,and kinetic characterization.Prep.Biochem.,18(4),459-471. 68.Ullah,A.H.J.and Gibson,D.M.1987.Extracelluall phytase(E.C.3.1.3.8)from Aspergillus ficuum NRRL 3135 : purification and characterization.Prep.Biochem.,17(1),63-91. 69.Uppstrom,B.and Svensson,R.1980.Determination of phytic acid in rapeseed meal.J.Sci.Food Agric.,31,651-656. 70.Vasilets,L.A.,Omay,HS.,Ohta,T.,Noguchi,S.,Kawamura,M.and Schwarz,W.1991.Stimulation of the Na+/K+ pump by external [K+] is regulated by voltage - dependent gating.J.Biol.Chem.,266(25),16285-16288. 71.Wang,H.L.,Swain,E.W.and Hesseltine,C.W.1980.Phytase of molds in oriental food fermentation.J.Food Sci.,45,1262-1266. 72.Wozenski,J.and Woodburn,M1975.Phytic acid(Myo-inositol hexaphosphate)and phytase activity in four cottonseed protein products.Cereal Chem.,52,665-669. 73.Xu,Pin.and Price,J.1992.Recent advances in methodology foranalysis of phytate and inositol phosphates in foods.Progess food Nutr.Sci.,16,245-262. 74.Zhou,J.R.,Fordyce,E.J.Raboy,V.,Dickinson,D.B.,Wong,M.S.,Burns,R.A.and Erdman,J.W.1992.Reduction of phytic acid soybean products improves zinc bioavailability in rats.J.Nutr.,122,2466-2473.