

Discussion on Measuring Method of Phytase Activity According to Term Enzyme Specificity

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Introduction

According to the term enzyme specificity, a discussion on measuring method of phytase activity used now showed that the method could not show the order of inorganic phosphates released from sodium phytate to be helpful to classify or group the phytases studied now, but it is still be useful in production units at the grassroots level and preliminary researches of the phytase. The term enzyme specificity could be useful to interpreting accurately the results got by measuring enzyme activity and to judging the advantages and disadvantages of the method to measure enzyme activity. The discussion on the term enzyme specificity here could also be used as an example for students to study and apply the term they met or will meet.

Enzyme has been deeply concerned and studied as an important class of biocatalyst and the term enzyme specificity is one of the basic concepts introduced in text books [1,2]. The accurate implication and expression of the term enzyme specificity was discussed, for the different understanding existed in different textbooks [3]. For example, in pentose phosphate pathway, sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate can not only react to become xylulose 5-phosphate and ribose 5-phosphate catalyzed by transketolase, but can also react to become erythrose 4-phosphate and fructose 6-phosphate catalyzed by transaldolase [1,2]. Though sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate are all the same substrates for transketonase and transaldolase, the reactions and the products catalyzed by the both enzymes are not the same respectively. So reacting specifically and producing the specific product(s) should be considered fundamentally and importantly when the term enzyme specificity is used to analyze an enzymatic reaction [3].

Phytase is the important enzyme used in food and feed industries [4], in remediation of polluted soils [5], in the improvement of plant growth and nutrition [6]. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) classifies phytases as either 3-phytase (EC 3.1.3.8), 6-phytase (EC 3.1.3.26) or 5-phytase (EC 3.1.3.72) depending upon the specific position of the initial dephosphorylation reaction [7]. Measuring phytase activity is the basic experiment during the researches. The method, having been used typically to measure phytase activity, has been based on measuring the released inorganic phosphates catalyzed by the phytase by a modification of the ammonium molybdate method [8]. To put it simply, phytase reaction mixture included some sodium phytate, acetate buffer or glycine-HCl buffer or citric acid-sodium citrate buffer of a certain concentration and with a certain pH and the crude enzyme solution of a certain concentration, and after its work at some temperature for a period, the phytase activity was determined by measuring the resulted inorganic phosphate with the ammonium molybdate solution [9,10]. But the method could not show the order of inorganic phosphates released from sodium phytate. For example, Greiner et al. [11] showed that the phytate-degrading enzyme P2 of *E. coli* dephosphorylates myo-inositol hexakisphosphate in a stereospecific way by sequential removal of phosphate groups

via D-Ins(1,2,3,4,5)P₅, D-Ins(2,3,4,5)P₄, D-Ins(2,4,5)P₃, Ins(2,5)P₂ to finally Ins(2)P (notation 6:1:3:4:5), using a combination of high-performance ion chromatography analysis and kinetic studies. Greiner et al. [12] showed that the phytate-degrading enzyme from baker's yeast dephosphorylates *myo*-inositol hexakisphosphate in a stereospecific way by sequential removal of phosphate groups via D-Ins(1,2,4,5,6)P₅, D-Ins(1,2,5,6)P₄, D-Ins(1,2,6)P₃, D-Ins(1,2)P₂, to finally Ins(2)P (notation 3/4/5/6/1), using a combination of high-performance ion chromatography analysis and kinetic studies. What do the facts mean? It shows that the reaction progresses catalyzed by the two phytate-degrading enzymes and the resulted intermediate products which exist stably and can be determined are not the same respectively although the both enzymes act on the same substrate sodium phytate and the produced end products such as Ins(2)P and inorganic phosphates are the same. So, strictly speaking, the two enzymes are not the same according to the term enzyme specificity. It is obvious that only measuring phytase activity cannot provide more information to study phytase further. Maybe this analysis can be useful to realize why the phytases can be classified as either 3-phytase (EC 3.1.3.8), 6-phytase (EC 3.1.3.26) or 5-phytase (EC 3.1.3.72). And maybe this analysis can also be useful to realize no matter whether the phytases can be grouped as histidine acid phytase (HAPhy), bpropellerphytase (BPPHy), purple acid phytase (PAPhy), or protein tyrosine phytase, as reviewed by Handa et al. [4], measuring phytase activity is only the most basic work. Considering the progresses and intermediate products in the phytate-catalyzing reaction, maybe it is better that phytases are a class of phosphatases which catalyze phosphate monoester hydrolysis of phytic acid in a stepwise manner, there by generating less phosphorylated *myo*-inositol derivatives and inorganic phosphate [13]. But it should be aware that the method used to measure phytase activity mentioned above has the advantages of low price and convenient use in production units at the grassroots level and preliminary researches of the phytase, for it grasps the generated inorganic phosphate in the reaction system no matter what less phosphorylated *myo*-inositol derivatives are generated.

As shown above, the term enzyme specificity could be useful to interpreting accurately the results got by measuring enzyme activity and to judging the advantages and disadvantages of the

method to measure enzyme activity. The discussion on the term enzyme specificity here could also be used as an example for students to study and apply the terms they met or will meet.

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