Animal Nutrition 2 (2016) 12–17

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Review article

Progress in the biological function of alpha-enolase

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ARTICLE INFO

Article history:
Received 16 December 2015
Accepted 3 February 2016
Available online 2 March 2016

Keywords:
α-enolase
Glycolysis
Plasma plasminogen
Cancer
Parasite

ABSTRACT

Alpha-enolase (ENO1), also known as 2-phospho-D-glycerate hydrolase, is a metalloenzyme that catalyzes the conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway. It is a multifunctional glycolytic enzyme involved in cellular stress, bacterial and fungal infections, autoantigen activities, the occurrence and metastasis of cancer, parasitic infections, and the growth, development and reproduction of organisms. This article mainly reviews the basic characteristics and biological functions of ENO1.

1. Introduction

Lohman and Mayerho discovered enolase in muscle extracts when studying the conversion of 3-phosphoglyceric acid into pyruvic acid in 1934, Subsequent studies have shown that three types of enolase isoenzymes exist in mammals: α-enolase (ENO1) is present in almost all mature tissues; β-enolase (ENO3) exists primarily in muscle tissues; and γ-enolase (ENO2) occurs mainly in nervous and neuroendocrine tissues. All enolases are composed of two identical subunits. The molecular weights of enolases range from 82 to 100 ku. In humans and other mammals, 3 independent genetic loci (α, β and γ) encode the 3 enolase isozymes. However, another enolase that is different from ENO1, ENO2 and ENO3 has recently been discovered in human and mouse sperm. This newly discovered enolase, termed enolase 4 (ENO4), is related to sperm motility and male reproduction (Nakamura et al., 2013). The present article mainly reviews the basic characteristics and biological functions of ENO1.

2. Basic characteristics of ENO1

Alpha-enolase, also known as 2-phospho-D-glycerate hydrolase, is a metalloenzyme that catalyzes the conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway. Petrak et al. (2008) statistically analyzed the frequency of the appearance of various terms related to human, rat and mouse proteins in the journal "Proteomics" (volumes from 4 to 6, from 2004 to 2006) and calculated the frequency of each term present in the database. It was found that ENO1 was a protein with an extremely high term frequency. Pancholi (2001) compared the amino acid sequences of ENO1 derived from 39 species. They found that although the amino acid sequences of ENO1 are different between species, ENO1 appears to be highly conserved. Alpha-enolase exhibits an overall amino acid sequence homology across species from 40% to 90% (Pancholi, 2001). Alpha-enolase proteins derived from various species are all composed of two structural domains: a smaller N-terminus and a larger C-terminus. The N-terminus shows a $\beta 3\alpha 4$ topology, while the C-terminus shows an $h\beta\beta\alpha\alpha$ (βa) 6 topology.

For most enolases, fluoride acts as an inhibitor, while Mg^{2+} is the most important metal activator. In yeast systems, metal cations and fluoride bind to enolase at the active center of the enzyme, forming a complex. The complex blocks the binding of substrates to the enzyme in yeast systems, thereby exerting an inhibitory effect.

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2016.02.005

Studies have also found that Mg^{2+} is an activator of *Leuconostoc mesenteroides* 512FMCM. In addition, Mn^{2+} and Zn^{2+} exhibit the same effect (Lee et al., 2006). Manganous ions shows a strong activating effect on the enolases of *Candida albicans* and yeast. However, the activating effect of Mn^{2+} on carp muscle enolase is rather weak. Compared with Mn^{2+}, Zn^{2+} exerts a much stronger activating effect on carp enolase but a weaker effect on yeast enolases and *C. albicans* enolases. The above discoveries indicate that distinct activators and inhibitors exist for different animal enolases.

Alpha-enolase is abundantly expressed in most cells. Alpha-enolase is abundant in the cytoplasm and is also present at the cell surface and in nuclei (Pancholi and Fischetti, 1998). Because of the conservative nature of glycolytic enzymes (including ENO1) across millions of years, this class of enzymes is generally considered rather "dull". Glycolytic enzymes have even been labeled "void of sophisticated regulatory functions" because only minor changes in the concentrations of the enzymes occur in the presence of external stimuli and the enzymes only play a catalytic role in certain reactions. However, unlike the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene, *ENO1* is not a housekeeping gene. The expression of *ENO1* changes with the occurrence of pathological changes in organisms and over the course of cell growth. Current studies show that, in addition to their glycolytic activities, the glycolytic enzymes play important roles in several biological and pathophysiological processes. Specifically, studies demonstrate that ENO1 is closely related to cancer, systemic fungal disease, odontopathy and autoimmune diseases.

3. Biological functions of ENO1

3.1. Alpha-enolase participates in stress responses

A number of studies have shown that ENO1 plays an important role in the development of stress responses. Proteomic studies on the mechanisms underlying the development of cellular stress responses have shown that ENO1 is one of the proteins that are expressed differentially before and after stress exposure. Data have shown that cells often express specific proteins (such as heat shock proteins [HSPs]) and glucose-regulated proteins (including ENO1) to adapt to high temperatures and glucose deprivation (Young and Elliott, 1989). Under conditions of chronic hypoxic stress, the expression levels of 5 cellular stress-related proteins (with molecular weights of 34, 36, 39, 47 and 57 ku) are significantly altered in mammalian endothelial cells. Moreover, the changes in protein expression levels are time and oxygen concentration dependent (Graven et al., 1993). It has been confirmed that the 47-ku protein expressed by endothelial cells is ENO1. It is speculated that ENO1 promotes anaerobic metabolism upon hypoxic stress, thereby exerting a protective effect on cells.

Studies in microorganisms have found that the expression of several HSPs is induced in microbes of the genus *Saccharomyces* upon exposure to high temperature stress. One HSP, HSP48, has been identified as ENO1. Studies have shown that during the cellular response to hypoxic stress, ENO1 acts as a stress protein and activates the expression of hypoxia-inducible factor-1 (HIF-1) (Aaronson et al., 1995), indicating that ENO1 may enhance the protective effect on cells through promoting anaerobic metabolism. The above results indicate that HSP48 is the expression product of the *ENO1* gene and is related to temperature tolerance and growth regulation in microorganisms. Iida and Yahara (1985) found that the expression of the *ENO1* gene is regulated by the heat shock resistance (*HSR1*) gene and that mutations in HSR1 render microorganisms extremely tolerant to high temperatures. These findings suggest that ENO1 is closely related to heat stress (Iida and Yahara, 1985). Although ENO1 is also an HSP, the stability of ENO1 varies with the thermal environment. It has been found that the ENO1 proteins of yeasts and streptococcal strains isolated from rats exhibit high thermal stability. In contrast, the thermal stability of ENO1 from carp muscles, rabbit muscles and bovine brain is rather poor (Kustrzeba-Wójcicka and Golczak, 2002).

Some researchers have found that extracellular signal-regulated kinase 1/2 (ERK1/2) achieves its functions in cardiomyocyte contraction and survival by regulating ENO1. This finding indicates that ENO1 enhances the contractile force of impaired cardiomyocytes in hypoxic conditions. Under the stimulatory condition of ischemic hypoxia, ENO1 restores cellular ATP levels and prevents the death of cardiomyocytes. Stress generated upon cardiomyocyte injury activates ENO1 in the cytoplasm. Activated ENO1 is transported to the cytoskeleton and contractile filaments to stabilize these structures via its molecular chaperone activity (Mizukami et al., 2004). In summary, ENO1 may play critical roles in a variety of stress responses. Such effects of ENO1 are essential for the survival of cells.

3.2. ENO1 and bacteria

Alpha-enolase exists as a cell surface protein in a variety of prokaryotic and eukaryotic organisms. In addition, ENO1 is capable of binding to plasminogen. Therefore, it is likely that ENO1 plays important roles in the development and progression of disease through regulation of the extracellular and intravascular fibrinolytic systems. Alpha-enolase is also related to many types of bacterial infections; research in this area has mainly focused on streptococcal and *Diplococcus pneumoniae* infections.

A study conducted by Pancholi and Fischetti (1998) showed that as a potent plasminogen-binding protein on the bacterial surface, ENO1 is related to the pathogenic effects of mucosal pathogen group A streptococci and *Diplococcus pneumonia*. In addition, it has been found that enolase on the surface of mucosal pathogen group A streptococci possesses a stronger capacity for binding to plasminogen in comparison with other surface proteins of streptococci (Berge and Sjobring, 1993).

In studying *D. pneumoniae*, researchers discovered that ENO1 is the only enolase present in this species. Lenz et al. (2003) found that ENO1 present on the surface of *Streptococcus pneumoniae* comes from inside the bacterial cell and is then secreted to the cell surface. However, some researchers believe that *S. pneumoniae* ENO1 is acquired from other cells that have undergone apoptosis or other forms of cell death (Adrian et al., 2004). In addition, ENO1 located on the surface of *D. pneumoniae* adheres to plasminogen, which is abundant in the human body and enhances the activity of plasmin, a critical step in *D. pneumoniae* infection (Bergmann et al., 2005). Alpha-enolase of *D. pneumoniae* promotes the plasmin-mediated degradation of the reconstituted basement membrane and simultaneously induces the formation of neutrophil extracellular traps (NETs) (Mori et al., 2012). However, these effects of ENO1 are not related to interleukin 8 (IL-8) and lipopolysaccharide (LPS). The above effects of ENO1 not only increase the activity of bacteria in human blood but also enhance the antigen recognition capability of neutrophils in the innate immune response. Kolberg et al. (2006) conducted a study on *S. pneumoniae* and showed that the binding of ENO1 to plasmin is sufficient to cause pneumonia, despite the low expression level of ENO1 on the surface of *Streptococcus pneumonia*.

Staphylococcal enolase is a laminin binding protein, suggesting that the enolase exerts its functions through pathways involving the binding of enolase to extracellular matrix proteins (such as fibronectin on the surface of gram-positive bacteria) (Pancholi and Fischetti, 1998). However, it is puzzling that despite the structural similarity between streptococcal surface ENO1 and staphylococcal