Identification of a Glycerophosphocholine Phosphodiesterase, GDE5, in chicken

Hao Gao¹, Pingan Chang^{1*}

(¹Chongqing Key Laboratory of Big Data for Bio-intelligence, College of Bio-information, Chongqing University of Posts and Telecommunications, Chongqing 400065, P. R. China)

Abstract: Glycerophosphodiester phosphodiesterase (GDPD/GDE) catalyzes the hydrolysis of glycerophosphodiesters to glycerol 3-phosphate and alcohol. It was discovered that the glycerophosphodiesterase family plays a role in lipid metabolism and signal pathway in recent years, but little has been known about the characteristics of chicken GDEs. Here, chicken GDE5 (cGDE5) was identified and characterized for the first time. The full length coding cDNA sequence of cGDE5 was cloned, which encoded a polypeptide with 678 amino acids containing a carbohydrate-binding module 20 (CBM20) and a GDPD domain. Tissue expression profiles showed that cGDE5 mRNA was high in various tissues, such as heart, brain, skeletal muscle and testis. Moreover, cGDE5 was demonstrated to exhibit glycerophosphocholine phosphodiesterase activity. These results together suggested that cGDE5, as a unique member of GDE family, may play multiple roles as a cytoplasmic glycerophosphocholine phosphodiesterase.

1 Introduction

Two important factors considered by the poultry industry are meat yield and quality. At present, chickens are marketed in about half the time and at about twice the body weight compared to 50 years ago, which is closely related to a significant increase in the muscle proportion^[1]. As a complicated life process, the growth and development of livestock is controlled by a significant amount of genetic factors. A lot of metabolites, including deacylated glycerophospholipids, the glycerophosphodiesters (GPs), have been investigated in adult skeletal muscles. Although the amounts of skeletal muscle GPs in human and mouse skeletal muscle tissues are reportedly altered in response to physical activity, the environment, and pathological conditions^[2-4], the biological function of skeletal muscle GP has not been studied to date.

Glycerophosphodiester phosphodiesterase (GDPD/GDE, EC3.1.4.46) catalyzes the hydrolysis of GPs to glycerol 3-phosphate (G3P) and alcohol^[5]. The GDE family proteins have attracted attention recently for their emerging physiological roles. GDEs are highly conserved proteins in prokaryotes and eukaryotes^[6]. Previous studies were mainly focused on mammalian GDE1-3 and showed they were involved in diverse physiological functions. GDE5 not only has a GDE domain, but also contains a carbohydrate-binding domain^[7]. Mouse GDE5 was initially identified to be a GPC phosphodiesterase and controls skeletal muscle development^[8]. GDE5DeltaC471 that contains GDE sequence but lacks GPC phosphodiesterase activity also innegatively

regulates skeletal muscle development^[9]. Moreover, adipose tissue weight and blood triglyceride levels were increased in the transgenic mice specifically expressing GDE5DeltaC471 in skeletal muscle^[10]. These results indicated GDE5 played a key role in muscle and adipose tissue development.

The chicken is not only an important animal for providing meat, but it is also serves as a valuable avian model. In contrast to mammals, there are only six chicken GDE members to be predicted until now. Little has been known about chicken GDEs, except that GDE2 was involved in neuron differentiation^[9,10]. In our study, chicken cGDE5 was first cloned and built a unique 3-D molecular model. Then *cGDE5* mRNA was found to express in various tissues. Finally, cGDE5 tagged with green fluorescence protein (GFP) was observed to be a cytological protein and identified to have GPC-hydrolyzing activity.

2 Results

2.1 Molecular cloning and analysis of cGDE5 gene coding sequence

The full length coding cDNA sequence of cGDE5 is 2037 bp (GenBank accession No. KC967655.1), and 678 amino acid polypeptide is encoded (Fig. 1A). There is 99% identity between the deduced proteins from the cloned and the predicted *cGDE5* gene (data not shown). The theoretical molecular weight and isoelectric point of cGDE5 are 76.9 kDa and 5.85 respectively, which were

* Corresponding author: * E-mail address: changpingan@aliyun.com.

determined by the Compute pI/Mw tool on the ExPASy server. Protein domain analysis showed that cGDE5 had a carbohydrate-binding module 20 (CBM20) (residues 3–105) in the N-terminal region and a GDPD domain (residues 324–612) in the C-terminal region (Fig. 1B). By the most conserved sequence comparison of cGDE5 with

mammalian identified GDEs, the three amino acid sites necessary for GDE activity are E359, D361 and H374 (Fig. 1A). However, unlike other GDEs, there is not any transmembrane region in cGDE5 as predicted by the TMHMM2 program.

Fig. 1 Analysis of the deduced cGDE5 amino acid sequence.

2.2 cGDE5 is a unique member of chicken GDE family

Because there is a 56-residue conserved region in the GDPD domain among all identified mammalian GDE proteins and the bacterial GDEs sequence^[5], a search of chicken expressed sequence tags (EST) database based on their amino acid homology is expected to find out all chicken GDEs cDNA sequences. However, there are only six chicken GDE members. By the comparison of the most conserved sequence in all chicken GDEs, it was found that the three amino acid positions E, D and H were the most conserved (Fig. 2A). The multiple sequence alignments indicate that although there are not

high similarity between cGDE5 and other chicken GDEs (data not shown), a high homology exists in the GDPD domain of chicken GDE protein family (Fig. 2A). Based on phylogenetic analysis of the GDE domain, we further classified the family into two distinct groups: one group contains GDE4 and GDE5, the other includes GDE1-3 and GDE6 (Fig. 2B). Domain-structure analysis showed that GDE1-4 and GDE6 appear to be membrane proteins and contain multiple putative transmembrane regions (data not shown). In contrast, GDE5 has not any transmembrane region but contains an N-terminal CBM20 (Fig. 1B). The diverse domain structures of these GDEs indicate that cGDE5 is a unique member in chicken GDE family.

Fig. 2 The GDE domain sequence alignment and phylogram of the chicken GDE family. A, Identical amino acids in all six proteins were marked with an asterisk (*), conservative substitutions with a colon (:), and semi-conservative substitutions with a period (.). B, The numbers indicate the bootstrap confidence values obtained for each nodes after 1000 replications.

2.3 Molecular 3D model of cGDE5 protein domains

To further study the relationship between structure and function, we generated molecular 3D models of cGDE5 CBM20 and GDPD domains respectively using SWISS-MODEL (Fig. 3). It showed a β -sandwich fold with eight β -strands distributed in two β -sheets and an immunoglobulin-like topology. This fold made an open-sided distorted β -barrel with six loops of significant length. CBM20 folded as an antiparallel beta-barrel

structure with two starch binding sites. By sequence alignment, binding site 1 of cGDE5 CBM20 consists of residue 32, 63, 86-87, 92. The indole rings of Trp32 and Trp86 form the central part of a carbohydrate binding platform (Fig 3A). By contrast, binding site 2, defined by residue 15,16-19,46,48, is more extended and has higher structural plasticity than binding site 1 (Fig 3B).

As shown in Fig. 3C, The model of the cGDE5 GDPD domain contains a 302 residue region (residues 316 - 617) by using *Silicibacter pomeroyi* glycerophosphoryldiester phosphodiesterase (Protein Data Bank code: 3112.1.A) as the template. The model

consists of a central β sheet made up of eight strands and 10 surrounding α -helices. The predicted α/β structure resembles a triose-phosphate-isomerase (TIM) barrel

domain. We further located the most probable three sites for catalytic activity, Glu359, Asp361, and His374, in the 3-D structure (Fig. 3C).

Fig. 3 Molecular 3-D model of the CBM20 (A and B) and GDPD (C) domains of cGDE5. A and B were the 3-D model of carbohydrate-binding site1 and 2 respectively. Side chains of the conserved carbohydrate-binding sites and the GDPD catalytic active sites were rendered in stick format.

2.4 Expression profiles of cGDE5 in adult chicken tissues

We used real-time quantitative PCR to detect the expression of GDE5 mRNA in chicken tissues to evaluate the physiological role of cGDE5. As shown in Fig 4, the highest mRNA level was observed in heart, and the transcript was also highly expressed in chicken brain, skeletal muscle and testis. In contrast, there were relative low levels in liver, kidney and adipose tissue.

Fig. 4 Expression profiles of *GDE5* gene in chicken various tissues.

2.5 Subcellular localization of cGDE5

We transfected cGDE5 labeled with GFP at the C-terminal and GFP into HEK293T cells to study the subcellular distribution of cGDE5. In HEK293T cells expressing GFP itself, intense fluorescence was distributed throughout the cytoplasm (Fig. 5). The result

showed that cGDE5 was a cytosolically-located protein.

Fig. 5 Distribution of GFP-tagged cGDE5 protein in mammalian cells.

2.6 GPC phosphodiesterase activity assay of cGDE5

Previous studies identified that mouse and human GDE5 were GPC phosphodiesterase, so cGDE5 may have the same catalytic activity. First, the expression of cGDE5-GFP was detected using the GFP antibody (Fig. 6A). Then an enzymatic assay using HEK293T cells overexpressing cGDE5-GFP showed that GPC phosphodiesterase activity was significantly increased in cGDE5-GFP expressing cells compared with GFP-expressing cells (Fig. 6B). In addition, there was no difference in protein stability between GFP and cGDE5-GFP (data not shown). These results indicated that GPC can be hydrolyzed by cGDE5.

Fig. 6 GPC phosphodiesterase activity of cGDE5 in mammalian cells. A, The expression of cGDE5-GFP in HEK293T cells. B, Assay of GPC phosphodiesterase activity.

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