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Minireview

Evolutionary conservation of biotinidase: Implications for the enzyme's structure and subcellular localization

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Abstract

Biotinidases from various species ranging from fungi and insects to human have specific amino acids, and regions that are evolutionarily conserved. These specific amino acids and regions are further supported by their homology to a variety of amidases and nitrilases and by the location of missense mutations that cause biotinidase deficiency in humans. Glu-Lys-Cys residues from three of these regions are considered the catalytic triad involved in the catalysis of the amide linkage. The last one-third of the biotinidase sequence is lacking in nitrilases–amidases, which do not bind biocytin or biotin, therefore, it is likely that the biocytin–biotin-binding site of biotinidase is within this portion of the molecule. Although there are many missense mutations at the far C-terminus of the enzyme, the function of this region is still unclear. Biotinidase may have different functions in different cells or in different subcellular compartments. Using computer programs that predict the subcellular localization of proteins based on their N-terminal signal peptides, microsomal localization resulting in secretion was predicted for biotinidase from all species, whereas there is little consistent support for mitochondrial or nuclear localization of the enzymes. Additional immunohistochemical studies of various human tissues at different stages of development are necessary to resolve the ambiguity of subcellular localization of biotinidase.

Keywords: Biotinidase; Evolutionary conservation; Subcellular localization; Signal peptides; Amidase; Nitrilase

Introduction

Biotin is the coenzyme for four carboxylases in humans, pyruvate, β-methylcrotonyl CoA, propionyl CoA, and acetyl CoA carboxylases [1]. Biotin holocarboxylase synthetase biotinylates the ε-amino group of a lysyl residue of each of the apocarboxylases, thereby forming active holocarboxylases. When the carboxylases are proteolytically degraded, the biotin remains bound to the lysine as biocytin (biotinyl-ε-lysine). The biocytin is subsequently hydrolyzed by biotinidase (EC 3.5.1.12) to biotin and lysine at acidic pH [2]. Biotinidase also has biotinyl-transferase activity at physiologic pH and can transfer biotin to suitable nucleophilic acceptor molecules, such as histones [3]. Biotinidase deficiency, an autosomal recessively inherited disorder, is characterized by neurological and cutaneous symptoms [3,4]. The disorder can be readily treated with oral administration of pharmacological doses of biotin. Biotinidase deficiency meets most of the criteria for newborn screening and is screened for in the majority of states in the United States and in over 25 countries [5].

To better understand the structure–function relationships of the enzyme and to determine the portions of the molecule involved in its various activities, attempts have been made to express sufficient quantities of the enzyme to crystallize the protein. However, possibly due to the

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fact that the enzyme is highly glycosylated, these attempts have so far been unsuccessful. Another less definitive method of determining regions or motifs in the enzyme is to assess the conservation of amino acids or amino acid sequences of the enzyme in various species and to compare them with those of related enzymes, such as amidases and nitrilases. In addition, the importance of these conserved amino acids or sequences can be further confirmed by assessing the location of missense mutations that cause biotinidase deficiency. We have shown previously that biotinidase is conserved in mammalian species [6], but we have refined this analysis by demonstrating those amino acids or sequences of biotinidase that are conserved evolutionarily with eukaryotes down to insects and fungi. This provides stronger evidence as to which amino acids and regions of the molecule are important for activity and function.

In addition, because the enzyme may have different functions in different cells or in different subcellular compartments, it is important to compare the N-terminal signal peptides that are formed in various species for clues to the subcellular localization of the enzyme. Using computer programs that predict the probability of N-terminal signal peptides of the biotinidases from various species, we have predicted likely subcellular localization(s) of the enzyme. This provides further insight into the potential activities and functions of the enzyme.

Methods

Protein sequences of biotinidases and putative biotinidases from various species were obtained by BLAST searches from the NCBI database as follows: human, *Homo sapiens*: NP_000051; monkey, *Pan troglodytes*: XP_526143; dog, *Canis familiaris*: XP_534255; mouse, *Mus musculus*: NP_079571; rat, *Rattus norvegicus*: NP_001012047; chicken, *Gallus gallus*: XP_418738; Amphibian, *Xenopus tropical*: NP_001011263; fish, *Takifugi rubripes*: AAO15005; *Drosophila melanogaster*: NP_572298; fungi, *Dictyostelium discoideum*: DDB0205 980. Human and *D. melanogaster* represent reviewed RefSeq records while mouse and rat are provisional records. Monkey, dog, and chicken sequences represent RefSeq models. Amphibian, fish, and fungi sequences are non-reviewed conceptual translations based on mRNA evidence.

Biotinidase activity has been determined in serum or tissues of all the animals described above except fungus.

The protein sequences of the various biotinidases were aligned using Clustal W: http://www.ch.embnet.org/software/ClustalW.html and displayed and compared using BoxShade: http://www.ch.embnet.org/software/BOX_form.html.

Missense mutations that cause profound and partial biotinidase deficiency have been reported previously [7]. A single amino acid determined to result in no loss of enzyme activity has also been included (391P>S).

Putative signal peptides and probable microsomal/secretory or mitochondrial localization of each of the enzymes were determined using Signal IPSORT: http://hc.ims.u-okyo.ac.jp/iPSORT/#predict and SignalP 3.0 Server: http://www.cbs.dtu.dk/services/SignalP/ programs.

Results

Biotinidase and putative biotinidase enzymes were found in various eukaryotes from mammals to insects and fungi (Fig. 1). There is considerable homology among these enzymes with conservation of specific amino acids and regions. Three specific motifs also were found in related nitrilase and amidase enzymes [8]. These include the amino acid regions of human biotinidase, FPE at positions 110–112, YRKHNL at positions 210–215, and FTCFDILFFDP at positions 243–253. The Glu-Lys-Cys residues from these three regions are considered the catalytic triad involved in the catalysis of the amide linkage [8].

The translated or putatively translated N-amino termini of these enzymes were examined using Signal IPORT and SignalP 3.0 programs to predict the signal peptide, its cleavage site and its predicted subcellular localization (Table 1). These results indicate that all of the biotinidases have signal peptide sequences of various sizes (19–28 amino acids) that are consistent with a microsomal localization/secretory mechanism. Although several of the other biotinidases have N-termini that contain multiple methionines, only in humans is there a peptide in the N-terminus that is consistent with mitochondrial localization. Although the longer 28 amino acid signal peptide sequence is consistent with microsomal localization or secretory function, statistically the shorter signal peptide sequence is predicted to be more probable (Fig. 2).

Using PSORT II, none of the N-terminal sequences predicted nuclear localization. The "classical" signal peptides for nuclear localization usually have clusters of basic amino acids or with histidines and prolines. We examine each of the N-terminus sequences for basic amino acid and histidine and proline domains (Fig. 3). Examination of several of the biotinidases does have some clustering of basic amino acids, such as in humans, monkey, mouse, bird, and fungus, whereas other species have few or no basic amino acids. Only humans have a signal with histidine (no proline residues) clusters.

Discussion

The biotinidase gene contains at least four exons, spanning over 21 kb of chromosome 3p25 [9]. The sequence of intron 1 was incomplete at 12.5 kb. The cod-