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Biotinidase: its role in biotinidase deficiency and biotin metabolism☆

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Abstract

Renewed interest in biotinidase, the enzyme responsible for recycling the vitamin biotin, initially came from the discovery of biotinidase deficiency in 1982. Since then, the elucidation of other activities of the enzyme, alternative splicing of the biotinidase gene and differential subcellular localization of the enzyme have prompted speculation and investigations of its other possible functions. The results of these studies have implicated biotinidase in aspects of biotin metabolism, specifically the biotinylation of various proteins, such as histones. Biotinidase may have an important regulatory role(s) in chromatin/DNA function.

Keywords: Biotinidase; Biotinidase deficiency; Biotin; Alternative splicing; Subcellular localization; Biotinylation

1. Biotin

Biotin is covalently attached to the various carboxylases by biotin holocarboxylase synthetase [1,2]. The carboxyl group of biotin is linked by an amide bond to an ε-amino group of a specific lysine residue of the carboxylase. Following the proteolytic degradation of carboxylases, the resulting biocytin (biotinyl-ε-lysine) or small biotinyl-peptides are cleaved by biotinidase (EC 3.5.1.12) at the amide bond, releasing lysine or lysyl peptides and free biotin, which can then be recycled [3,4] (Fig. 1). Biotinidase may also play a role in the processing of dietary protein-bound biotin, thereby making the vitamin available to the free biotin pool [5]. We have shown that biotinidase can be biotinylated and can potentially function as a biotin-carrier protein. In addition, the enzyme, as a biotinyl-transferase, can biotinylate specific proteins or small acceptor molecules, which, in turn, may have physiologic functions [6,7] (Fig. 1).

2. Biotinidase deficiency

Biotinidase deficiency (OMIM 253260) is inherited as an autosomal recessive trait [8,9]. Individuals with profound biotinidase deficiency have less than 10% of mean normal activity in serum. Untreated individuals with profound deficiency may exhibit neurological and cutaneous features, including seizures, hypotonia, skin rash or alopecia, developmental delay, conjunctivitis, visual problems, such as optic atrophy, and hearing, accompanied by ketolactic acidosis and organic acidemia. All children with the disorder who have been treated with pharmacological doses of biotin (5–20 mg daily) improve clinically. The hearing loss, visual abnormalities and degrees of developmental delay do not appear to be reversible once they occur even with biotin therapy. Some children with profound biotinidase deficiency first develop symptoms later in childhood or during adolescence [10] and exhibit motor limb weakness, spastic paresis and eye problems, such as loss of visual acuity and scotomata, rather than the more characteristic symptoms observed in young untreated children with the disorder.

Neonatal screening for biotinidase deficiency [11,12] is conducted in many states in the United States and in many

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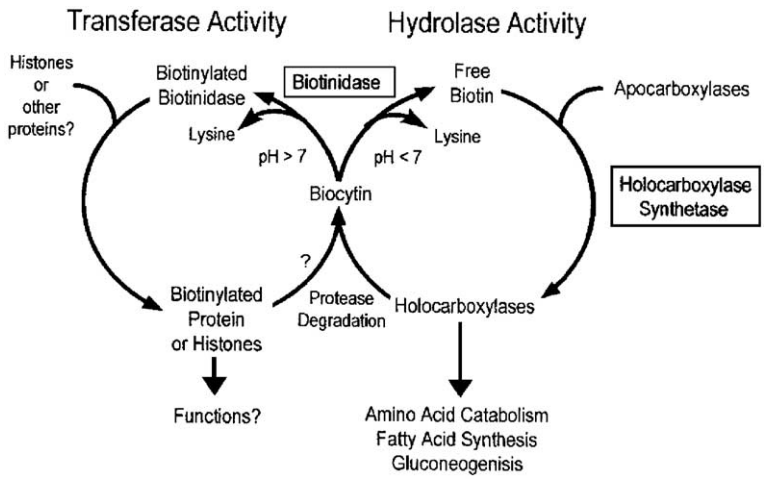

Fig. 1. The biotin bi-cycle. The 2 cycles include the biotinyl-hydrolase activity and biotinyl-transferase activity.

countries [13]. The estimated incidence of biotinidase deficiency is about 1 in 60 000.

2.1. Structure of biotinidase and the organization of the gene for biotinidase

Human biotinidase gene (BTD; 609019) consists of four exons [14]. Promoter features of the gene are consistent with the ubiquitous expression of biotinidase with characteristics of a CpG island, lack of a TATA element, six consensus methylation sites and three initiator sequences, thought to be important in transcription initiation of TATA-less promoters.

The cDNA that encodes for normal human serum biotinidase has been cloned [15] and encodes for a protein of 543 amino acid residues (56 661 Da). The serum enzyme has six glycosylation sites and is highly sialylated. Human serum biotinidase contains tyrosine and tryptophan motifs that are found in biotin-binding proteins, such as avidin and streptavidin [15].

There are amino acid homologies among human biotinidase (an aminohydrolase), bacterial aliphatic amidases and some bacterial and plant nitrilases [16]. One of the regions of homology contains the cysteine involved in the active site of aliphatic amidases and nitrilases. This suggests that cys_{245} of biotinidase is likely the cysteine in the active site of the enzyme and is the site of thioester formation that is integral for enzyme function. In addition, because the slightly upstream sequence, $YRK_{210-212}$, is also highly conserved among the three enzyme families, this region is likely essential for enzyme activity. There are now more than 100 known mutations that cause profound biotinidase deficiency [17,18].

3. Other potential functions of biotinidase

Until recently it was assumed that the only role of biotinidase is to cleave biocytin, thereby recycling biotin. Our recent studies suggest that serum biotinidase may be a biotin-carrier protein, retaining bound biotin as an acyl-enzyme, and possibly a biotinyl transferase capable of biotinylating specific proteins or small acceptor molecules.

3.1. Biotinylation of biotinidase

The results of studies examining biotinidase or other serum proteins as biotin-binding proteins are equivocal. Equilibrium dialysis studies of biotinidase with radiolabeled biotin at pH 7.4 suggest that biotinidase noncovalently binds biotin and may function as a biotin-carrier protein in plasma [19]. Other studies performed with plasma dialyzed against water (pH 6 or below) found that most of the biotin in serum was not protein bound [20]. Our finding of biotinylation of biotinidase in the presence of biocytin, but not biotin, is the only study that used biocytin as the substrate [21]. Binding of biotin to the enzyme likely occurs during the cleavage of biocytin [22]. If biotinidase acts as the biotin carrier protein in plasma, it may be responsible for transporting biotin into cells through a specific cell membrane receptor. Because biotinidase is highly sialylated, this may occur through desialylation of the enzyme and subsequent reaction with a galactose receptor on the cell membrane resulting in internalization of the biotinyl enzyme. Another possibility is that the biotinyl biotinidase reacts with a specific biotin receptor on the cell membrane [23]. Once the biotinyl-biotinidase enters the cell the biotin is either released by hydrolysis or transferred to appropriate cellular acceptors, depending on the pH of the compartment.

3.2. Biotinylation of histones

We have shown that biotinidase in the presence of biocytin, but not biotin, can biotinylate histones specifically [7] (Fig. 1). The K_m value for hydrolysis of biocytin by biotinidase is in the micromolar range and hydrolysis occurs optimally at pH 5.5–6, with a precipitous decrease in activity above pH 7. On the other hand, biotinylation of histones by biotinidase occurs at physiological pH and concentrations of biocytin (nanomolar range) [7]. The

discovery of biotinyl-transferase activity of biotinidase may explain several functions of biotin that are not due to its action as a coenzyme for the carboxylases.

3.3. Biotinylation of serum proteins

In addition to histones, we have found that myelin basic protein and polylysines are substrates for biotinylation. These results suggest that the biotinylation by biotinidase and certain proteins is not random. The function of this biotinylation is unknown.

4. Characterization of biotinidase transcription in tissues

We have identified and characterized three human biotinidase mRNA splice variants [24]. The cDNAs corresponding to these variants, 1a, 1b and 1c, are expressed in approximately equal relative quantities. The testis is the only tissue that expresses variant 1c. Comparison of the sequences of these mRNAs to the genomic sequence of biotinidase indicates that the variants likely result from alternative splicing at the 5′ end of biotinidase pre-mRNA. Depending on the site of translation, each splice variant could encode a distinct signal peptide, which may result in the tissue-specific intracellular localization of biotinidase.

Variant 1a produces a putative 41 amino acid signal peptide that could result in the localization of biotinidase to the nucleus, mitochondria and/or endoplasmic reticulum. Variant 1b could also produce either a 21-amino-acid signal peptide or a slightly longer 30-amino-acid signal peptide. We compared the expression of both the 21- and 41-amino-acid signal peptides with a third lacking any signal peptide in baculovirus/insect cells [25]. Both the 21- and the 41-amino-acid signal peptide were capable of secreting an active enzyme, whereas the construct without the signal peptide sequence failed to produce a secreted enzyme.

5. The cell biology of biotinidase

Our studies indicate that serum biotinidase activity correlates positively with the concentration of serum albumin in sera of individuals with cirrhosis [26], suggesting that serum biotinidase originates principally from the liver [4,27]. We have shown that the enzyme is sialylated in serum and is asialylated in tissues with secretory function, such as liver, pancreas [4,28], pancreatic juice and isolated zymogen granules [5]. These results are consistent with biotinidase being a secretory enzyme. This is consistent with our results [24,29] and those of others [4,30] indicating that the majority of biotinidase is enriched in the microsomal fractions of fresh differentially centrifugated fractionated human liver.

Fractions enriched for lysosomal or mitochondrial marker enzyme activities do not have much biotinidase activity. Western blot analysis of the same gradient fractions demonstrated the presence of an 85-kDa biotinidase protein, similar in size to the serum enzyme. Although biotinidase activity was not present in the fractions enriched for the mitochondrial enzyme marker activity, a 48-kDa, biotinylated, aglycosylated protein that cross-reacted with the biotinidase antibody was found in these fractions. To confirm the localization of the 48-kDa biotinidase protein, mitochondria were purified from rat liver by differential centrifugation and treated with digitonin to remove the outer membranes and to pellet the pure inner membranes (mitoplasts). Biotinyl-hydrolase and -transferase activities were present in the extracts of whole mitochondria, but not in extracts of the inner mitochondrial membranes. This suggests that the biotinidase activity identified in mitochondrial fractions is due to contamination of the enzyme in the whole preparation, and that hydrolytic activity of the enzyme is not located primarily in mitochondria. When whole mitochondria were separated into mitoplasts and outer membranes, the 48-kDa protein was identified only in the mitoplasts and the 85-kDa protein only in the outer membranes. The 48-kDa protein in mitochondria may be an internal alternative splicing product of biotinidase pre-mRNA.

With the exception of a single report that biotinidase localizes to the nucleus [31], most other studies have neglected to examine the nucleus for biotinidase activity even though there is strong evidence that biotin localizes to the nucleus in biotin-deficient states [32]. It was not until our report that biotinidase can biotinylate histones [7], which has been recently confirmed by others [33,34], that a possible role for biotinidase in the nucleus was considered. Preliminary evidence suggests that biotinylation and debio-

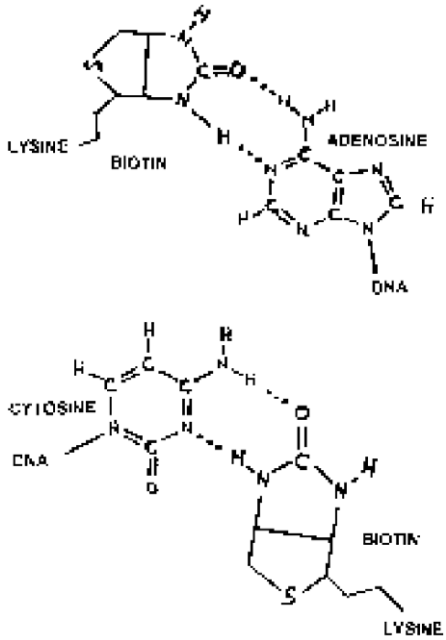

Fig. 2. Possible interactions between biotin or biotinylated proteins and nucleotides in DNA (or RNA).

tinylation of histones have a regulatory role in the cell [33,34], such as in DNA repair [35]. Furthermore, biotin holocarboxylase synthetase has been localized to the nucleus [36]. Rat liver homogenate was separated and the fractions collected [24]. Using discontinuous Percoll-metrizamide gradient of rat liver homogenate, approximately 60% of the biotinyl-hydrolase activity is retained in the postnuclear supernatant; the remainder is found in the pelleted nuclei and cell debris obtained by low-speed centrifugation of the whole-cell homogenate. Although preliminary studies using immunohistochemical techniques indicated that biotinidase is not found in the nuclei of HepG2 and fibroblast cells, it has been found in the nuclei of other cells at various times of cell differentiation (Wolf et al. unpublished data).

It is possible that biotinylation of histones causes various histones to bind or to be released from DNA, similar to that of other posttranslational modifications of histones, such as acetylation. On the other hand, we speculate that biotin or biotinylated histones, unlike those modifications, can directly interact with chromatin or DNA. We propose that hydrogen bonding can occur between the uriedo group of biotin or biotinylated histones and the nucleoside groups of thymine and cytosine in DNA or RNA, thereby regulating gene function (Fig. 2).

6. Summary

Biotinidase is likely differentially expressed in tissues. The enzyme or related proteins may be targeted to various subcellular localizations, including the nucleus. If this is confirmed, the enzyme may have multiple functions, including regulating chromatin and DNA function. These functions will be important implications for understanding genotype/phenotype correlations in biotinidase deficiency.

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