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Biotin-dependent regulation of gene expression in human cells☆

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Abstract

The role of biotin as cofactor of carboxylases and its importance in metabolic homeostasis are well known. In recent years, different researchers have suggested the participation of biotin as a regulator molecule in the control of gene expression. Biotin-dependent gene expression requires of the transformation of biotin into biotinyl-5′ -AMP by holocarboxylase synthetase and the activation of soluble guanylate cyclase and a cGMP-dependent protein kinase. The regulatory role of biotin is responsible for the correct expression of enzymes involved in biotin utilization in human cells. We propose that this mechanism protects the brain from biotin deficiency.

Keywords: Biotin; Holocarboxylase synthetase; Carboxylase; Vitamin utilization; Gene expression

1. Biotin and metabolism

The vitamin biotin is an essential nutrient for all living organisms because of its role as cofactor of enzymes involved in carboxylation and decarboxylation reactions [1]. In human cells, there are five biotin dependent carboxylases that catalyze key reactions in gluconeogenesis, amino acid catabolism and fatty acid synthesis [1,2]. During evolution, eukaryotic organism lost the ability to synthesize biotin, and therefore, their vitamin demands must be satisfied through the diet. However, in nature, biotin is present in trace amounts and most of it is not readily available by being covalently bound to proteins [1]. To cope with these problems, mammals evolved a complex mechanism known as biotin cycle to optimize biotin utilization. The biotin cycle is formed by three different proteins. In hepatocytes, the first protein in the biotin cycle is the sodium-dependent multivitamin transporter, which is responsible for the biotin transport across the cell membrane [1–4]. The second step of the cycle is catalyzed by holocarboxylase synthetase (HCS), an enzyme responsible for the transformation of biotin into biotinyl-5′ -AMP (B-AMP) and its transfer to carboxylases [1,2]. The last participant in the biotin cycle is the enzyme biotinidase responsible for the release of biotin from biocytin (biotinlysine) or biotinylated peptides derived from cell endogenous carboxylases turnover or intestinal digestion [1,2].

The delicate balance in utilization and recycling of biotin can be disrupted by genetic disorders with devastating consequences for metabolic homeostasis. In humans, HCS or biotinidase deficiency produces the neonatal or juvenile forms, respectively, of the disease multiple carboxylase deficiency (MCD). Multiple carboxylase deficiency patients are characterized by the reduction of all carboxylases activities, organic acidemia, hyperammonemia, dermatitis, alopecia, seizures and neurological damage. Although the two disorders are potentially lethal, their clinical and biochemical manifestations, except neurological damage, can be reversed by the administration of pharmacological doses of biotin [1,2].

2. Biotin-dependent gene expression

For the past 36 years, different research groups have presented evidence indicating that biotin is also required for the control of gene expression in laboratory animals and

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cells in culture. The list of proteins affected by biotin includes enzymes involved in glycolysis and gluconeogenesis, biotin metabolism, vitamin transporters and transcription factors [2,4–12]. The mechanism responsible for this nonclassic role of biotin remained elusive for many years.

To characterize the role of biotin in gene expression, our laboratory focused on the effect of biotin deficiency on the mRNA levels of enzymes from the biotin cycle in human hepatic cells. Incubation of HepG2 cells for 15 days in a biotin-free medium resulted in a gradual reduction (70–80% final reduction) in the mRNA levels encoding HCS, PCC, PC, ACC-1 and SMVT. The physiological impact of the decrease in HCS and SMVT mRNA levels was evidenced by the fact that biotin deficiency also reduced the amount of both SMVT and HCS proteins [10,16]. Addition of biotin to the deficient cell cultures resulted in the complete restoration of mRNA levels to normal values, demonstrating the specificity of the biotin effect [6,10]. Our results suggested that biotin maintains the expression of enzymes involved in utilization of exogenous biotin while having no effect on expression of biotinidase [10].

Biotin-deficient HepG2 cells responded to biotin or cGMP stimulation with a similar nonsynergistic increase in mRNA levels. These results led to the finding that transcriptional regulation of genes from the biotin cycle is mediated by a signal transduction pathway involving a soluble guanylate cyclase (sGC) and a cGMP-dependent protein kinase (PKG) [13,14]. This pathway was previously shown to be responsible for the biotin-dependent post-transcriptional regulation of ASGPR expression by Stockert et al. [7,8,12]. To continue the characterization of biotin-dependent gene expression, we used cells from patients with neonatal MCD. Mutations in the HCS gene produce a protein with Km values for biotin 370 times higher than normal cells [15]. In these studies, we showed that HCS-deficient cells require 100 more biotin than normal cells to increase mRNA levels suggesting that B-AMP, but not biotin, is the transcriptionally active form of the vitamin in human cells.

3. Paradoxical regulation of biotin utilization

Regulation of biotin cycle through the HCS-sGC-PKG pathway seems paradoxical in nature by apparently reducing the chances of cell survival under certain circumstances. This model suggests that low biotin concentrations would reduce the expression of enzymes of the biotin cycle decreasing the ability of cells to utilize the remaining vitamin from the surrounding medium.

It is safe to assume that during human evolution, biotin deficiency was not a rare event because of the low abundance of this vitamin in nature and the harshness of preagricultural life. Given these conditions, what is the physiological role of biotin-dependent transcriptional regulation? To answer this question, we made use of an animal model for biotin deficiency developed in the laboratory of Antonio Velázquez [9,13]. Wistar rats fed a biotin-free diet for up to 8 weeks were used to analyze the biotin cycle mRNA expression in different tissues. Our results revealed that biotin deficiency reduced the mRNA levels of HCS, SMVT and biotin-dependent carboxylases in liver and kidney. However, in the brain, the expression of these mRNAs was not affected, suggesting that this organ is privileged during biotin starvation. Previous reports confirm this hypothesis by showing that biotin deficiency reduces the activity and mass of biotin carboxylases in the liver and kidney, but not in the brain. Interestingly, biotinidase mRNA levels, as observed in human HepG2 cells, were not affected in any tissue, indicating that endogenous biotin recycling is not under the control of the HCS-sGC-PKG pathway. However, in the brain, biotinidase mRNA showed a 30% increase with respect to control animals, which may reflect an increase in the ability of this organ to reutilize endogenous biotin during nutritional stress.

We proposed that the role of the HCS-sGC-PKG pathway is to limit the demand of biotin in peripheral tissues by reducing its uptake and utilization. This system allows a continuous biotin supply to the brain where biotin enzymes are essential for neural function and development through anaplerosis of Krebs cycle and restoration of α-ketoglutarate because the release of glutamate and γ-aminobutyric acid from neurons and glutamine are exported from glia.

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