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Biotin—a regulator of gene expression☆

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Abstract

The role of biotin as the prosthetic group of the four biotin-dependent carboxylases in higher organisms is well recognized. Based on the roles of these carboxylases in metabolism, the requirement of biotin for cell viability, growth and differentiation was established. Biotin seems to have a role in cell functions other than as the prosthetic group of biotin enzymes. Biotin seems to influence processes such as the proliferation of the mesenchyme, spermatogenesis and song-bird vocalization. A direct effect of biotin, at the transcriptional level, has been shown for the key enzymes of glucose metabolism. Glucokinase, a key glycolytic enzyme, and phosphoenolpyruvate carboxykinase (PEPCK), a key gluconeogenic enzyme, are regulated in opposite directions by biotin in a manner similar to the action of insulin.

Keywords: Biotin; Gene expression; Key enzymes of glucose metabolism; Insulin

1. Introduction

Ligand–receptor systems constitute an intra- and intercellular communication system in which exogenous or endogenous molecules convey signals through association with membrane structure. Only a small percentage of the genome in eukaryotes is transcriptionally active at any particular time. Response elements, mostly upstream from the structural genes affected, regulate the rate of transcription of individual genes. Gene expression can be regulated at any of the steps in the pathway from DNA to RNA to protein. The regulation of gene expression during cellular differentiation and also through the influence of hormones, cytokines and growth factors, is well established. Most of the cells in multicellular organisms are capable of altering the pattern of their gene expression in response to extracellular signals. Among these signals should be included the nutritional status of the organism as this impinges on various regulatory systems. In addition to such indirect routes, certain dietary constituents influence gene expression by interacting directly with regulatory elements in the genome leading to changes in the rate of transcription of a given gene. The regulation (up or down) of transcription is mediated by alteration in the availability of transcription factors through changes in the factor's ability to bind to specific regulatory sequence motifs.

The physiological effects of vitamins follow their entry into the cell and subsequent transformation and association with specific apoenzymes resulting in an accentuation of a metabolic pathway. Where the vitamin function is other than as a coenzyme or a prosthetic group of an enzyme, its effect is through regulation of cellular protein synthesis. Lipid soluble vitamins such as vitamin A and vitamin D have been recognized to regulate gene expression by mechanisms similar to those of steroid hormones. Water-soluble vitamins such as biotin also seem to have a modulatory effect on gene expression. This review explores the function of biotin in the regulation of gene expression, based on evidence for the requirement of biotin for cell survival and cell differentiation as well as the requirement of biotin for developmental processes. In these instances, biotin has been related only to a

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process without a direct link to expression of specific DNA sequences. However, there is also evidence connecting biotin directly to the expression of specific genes.

2. Biotin requirement for cells in culture

The best-known and understood role of biotin is as the prosthetic group of the four-biotin containing carboxylases in higher organisms [1,2]. Biotin is covalently bound to a lysine residue of the carboxylase protein in acetyl-CoA carboxylase, propionyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase and pyruvate carboxylase. This explains its obligatory involvement in the metabolism of carbohydrates, lipids and deaminated residues of some amino acids and, hence, the requirement for biotin by all cells. However, various earlier studies indicated that cells in culture such as the HeLa cells do not require biotin, and it was further suggested that transformed cells might have the ability to synthesize biotin [3]. Using biotin-depleted fetal bovine serum (FBS) and Eagle's minimal essential medium, we demonstrated a requirement for biotin by HeLa cells, human fibroblasts and Rous sarcoma virus-transformed baby hamster kidney cells [4–7]. This was based on the viability, biotin content and activities of biotin-dependent and biotin-independent enzymes. Under cell culture conditions, it has been suggested that biotin is required for fatty acid synthesis and hence membrane formation. Under our experimental conditions, serum lipids were available for uptake by cells and, yet, a biotin requirement was demonstrated. We also showed that there was a significant decrease in the incorporation of leucine into protein of the homogenate or cytosol of biotin-deficient HeLa cells compared with cells grown in a biotin-supplemented medium. When biotin was added to the biotin-deficient medium, there was a twofold increase in the incorporation of leucine into protein [7].

Mammalian cultured cells, when they do not receive specific signals, which include specific growth factors, come to a halt in a quiescent nongrowing variant of the G1 state, referred to as G0. The synthesis of components of the cell-cycle control system is switched off. Normal cells in G1 arrest due to serine starvation start incorporating [^{3}H]thymidine into DNA as soon as serine is restored to the medium. Biotin-deficient HeLa cells under similar conditions do not incorporate [^{3}H]thymidine into DNA even when serine is restored to the medium. However, within 4 h of supplementation of biotin to the biotin-deficient medium, the incorporation of [^{3}H]thymidine into DNA reaches a maximum [6,7]. By this time there is a stimulation of protein synthesis. The two phenomena are related, and the growth-promoting effects might be achieved through synthesis of certain proteins. A requirement for high-density lipoprotein (HDL) has been shown from the growth of Madin–Darby canine kidney cells grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with transferrin. The components of the medium responsible for support of growth in the absence of HDL include biotin. Our results [6] indicate that the addition of biotin to the culture medium of biotin-deficient HeLa cells results in increased protein synthesis and increased peptidyl-puromycin formation. Progression through cell cycle is dependent on the synthesis of specific proteins, and the growth-promoting effect of biotin might be through stimulation of specific proteins.

3. Cell differentiation

The L1 subline derived from 3T3 mouse fibroblast cell line has the capacity to differentiate in the resting state into adipocyte cell type. When the cells reach confluence and start to differentiate, they greatly increase the rate of triglyceride synthesis. The increase in the lipogenic rate parallels the coordinated increase in the activities of the key enzymes of lipogenic pathway. This increase correlates with a marked rise in the nuclear run-off transcription rates for these mRNAs during differentiation [8]. The process of differentiation can be accelerated by increasing the amount of serum in the culture medium or by adding insulin or biotin. It took 24–48 h after the addition of biotin to the medium before deposition of triglyceride. This delay suggests that some factor required for the induction of the whole set of lipogenic enzymes (which, with the exception of acetyl-CoA carboxylase, are not biotin enzymes) might be formed under the influence of biotin. In rats made biotin-deficient, hepatic propionyl-CoA carboxylase (PCC) was less than 20% that in control rats, and it took at least 24 h after the addition of biotin for PCC to reach control activity and biotinylation levels [9]. If biotin were required only for the biotinylation of the apocarboxylase, the effect of biotin would be rapid as seen in the restoration of the activity of pyruvate carboxylase at the earliest time.

4. Palatal process

Congenital malformations have been reported in domestic fowl maintained on a biotin-deficient diet. Maternal biotin deficiency is teratogenic in mice even when the dams do not show any signs of biotin deficiency [10]. At mid-gestation, biotin-deficient embryos weighed less than normal embryos and had external malformations such as micrognathia and micromelia. There was a marked decrease in the size of the palatal process on day 15.5 of gestation, which may be due to altered proliferation of the mesenchyme. We investigated the development of the palatal process in culture [11]. After 72 h of organ culture, > 90% of the explants from normal mouse embryos (biotin replete) were at stage 6 of development. The corresponding figure for explants from biotin-deficient embryos cultured in a biotin-deficient medium was 6.5%. If the deficient explants were cultured in a medium containing biotin (10^{-8} M), the percentage at stage 6 rose to 30%. Administration of biotin to biotin-deficient dams 24 h prior to removal of the embryos resulted in 33% of the explants at stage 6 of development when cultured in a biotin-deficient medium and >50% at stage 6 if

cultured in a medium containing biotin (10^{-7} M). There was no detrimental effect of any of the organic acid intermediates or secondary metabolites on palatal closure of the explants when the compounds were added to the organ culture medium at a concentration of 10^{-4} M. These results highlight the continuous requirement for biotin during the proliferation of the mesenchyme, perhaps for the synthesis of growth factors during organ genesis.

5. Spermatogenesis

Early literature on biotin contains reports of delayed spermatogenesis and decreased number of spermatozoa due to biotin deficiency. In mammals, spermatogenesis is dependent primarily upon testosterone, which is produced by Lydia cells and acts on sterol and per tubular cells of the somniferous tubules to drive spermatogenesis. We have shown [12] that testicular and serum levels of testosterone are decreased in the biotin-deficient rat. Biotin deficiency was accompanied by a significant degree of sloughing of the seminiferous tubule epithelium in these rats. Treatment of biotin-deficient rats with gonadotrophins or biotin increases the serum levels of testosterone. However, even when testosterone levels are maintained at high levels in biotin-deficient rats by testosterone implants, the increase in serum testosterone does not result in normal spermatogenesis. The administration of biotin alone or biotin in addition to testosterone to biotin-deficient rats leads to normal spermatogenesis, thus suggesting that biotin might be involved in the formation of local testicular factor(s) which are required in addition to testosterone and follicle-stimulating hormone for the normal interaction among Leydig, Sertoli and peritubular cells. The identity of these proteins is not known, although we have shown that biotin is required for the synthesis of testicular proteins.

6. Neurotropic factor

Brain-derived neurotrophic factor is a member of the family of cell signaling molecules (neurotrophins), which have an important role in neuronal development and plasticity. Brain-derived neurotrophic factor has been implicated in development and adult plasticity within telencephalic brain regions that control learned vocal behavior in songbirds. Brain-derived neurotrophic factor expression seems to correlate with specific stages of songbird vocal learning. High levels of biotin have been reported in specific telencephalic nuclei (RA and HVC) among juvenile males [13]. It is possible that there may be a specific up-regulation of biotinylated proteins within RA and HVC nuclei in juvenile males. The developmental expression of this is correlated with vocal learning. High levels of biotin in the hippocampus, a brain region important for learning and memory, has also been reported [14], emphasizing the important role that biotin-regulated mechanisms might play in neuronal plasticity.

7. Specific protein synthesis

In addition to establishing a role for biotin in cellular process such as survival, differentiation and development, other evidences lead to the identification of a role for biotin in the regulation of the synthesis of specific proteins. Biotin holocarboxylase synthetase (HCS) adds biotin covalently to the apocarboxylases. Holocarboxylase synthetase mRNA is significantly reduced in the biotin-deficient rat and is increased following biotin administration to biotin-deficient rats, reaching control levels 24 h after biotin administration [15]. These results indicate the possibility of regulation by biotin of the genetic expression of the proteins involved. A regulatory role for biotin in the control of biotin HCS and carboxylase mRNA levels via signaling cascades involving HCS, guanylate cyclase and cGMP-dependent protein kinase has been proposed. Biotin deficiency has been shown to reduce the expression of SLC19A3, a potential biotin transporter, in leukocytes from human blood [16].

The asialoglycoprotein receptor is characteristic of fully differentiated hepatocytes. The human hepatoblastoma line HepG2 expresses maximum receptor activity only in confluent cultures. HepG2 cells grown to confluency in minimal essential medium made 10% with respect to FBS demonstrate an asiloglycoprotein receptor with ligand-binding characteristics and molecular mass comparable to those of the receptor purified from human liver. When the low-molecular weight fraction of FBS was removed, the expression of asialoglycoprotein receptor was significantly reduced. This was restored by the addition of biotin to the medium [17]. A biotin-dependent post-transcriptional event was suggested to permit the expression of the asialoglycoprotein receptor by HepG2 cells.

8. Enzymes of glucose metabolism

The enzymes of glucose metabolism seem to be very specifically regulated by biotin. Glucokinase, an adaptive enzyme is under dietary, nutritional and hormonal regulation. Similarly, phosphoenolpyruvate carboxykinase (PEPCK) is a key gluconeogenic enzyme under corresponding dietary, nutritional and hormonal controls. Both these enzymes are regulated in opposite directions by biotin in a manner similar to the action of insulin. It is to be noted that biotin is not a part of these enzymes.

9. Glucokinase

Earlier work from our laboratory demonstrated that liver glucokinase activity was altered in response to the biotin status of rats [18,19]. Biotin also plays a role in the precocious development of glucokinase in young rats [20]. Further work indicated that pharmacological levels of biotin increased the activity of glucokinase in biotin-replete animals [19]. Hepatic glucokinase of biotin-injected starved

rats had increased almost threefold in comparison with the levels in starved rats. The relative amounts of glucokinase mRNA in the liver of biotin-injected starved rats were increased fourfold over the levels seen in normal fed rats and almost 20-fold that seen in starved rats not receiving biotin injection. The induction of glucokinase mRNA by biotin is quite marked, relatively rapid and correlated with the increase in glucokinase enzyme activity. In "run-on" transcription experiments using isolated liver nuclei, biotin administration to the whole animal increased glucokinase gene transcription by the isolated liver nuclei by about sevenfold. This increased transcription of glucokinase was not simply due to an increase in overall transcriptional efficiency in as much as the transcription of the b-actin gene was unaffected [21].

Biotin has been shown to stimulate glucokinase activity in rat pancreatic islets in culture [22]. In islet cells treated with biotin (10^{-6} M), glucokinase mRNA levels increased by 140% and 180% over untreated cell levels, after 12 and 24 h, respectively. Treatment with biotin also increased insulin secretion. Islet glucokinase activity and mRNA levels were reduced by 50% in biotin-deficient rats. Insulin secretion in response to glucose was also impaired in islets from biotin-deficient rats indicating that the biotin effect on insulin release might be through glucokinase, which is the glucose sensor and metabolic signal generator in pancreatic B cells. All-trans retinoic acid exerts a stimulatory effect on hepatic glucokinase independent of the stage of maturity of hepatocytes [23].

10. Phosphoenolpyruvate carboxykinase

In both fasted and diabetic rats, hepatic PEPCK activities are markedly increased. Refeeding a high carbohydrate diet to fasted rats decreased PEPCK mRNA, which, in turn, is due to the repression of transcription of PEPCK gene by insulin. Three hours after biotin administration to starved rats, hepatic PEPCK mRNA levels decreased to 15% of the non-biotin-injected starved levels. Again, the effect of biotin paralleled the effect of insulin in these animals. In run-on transcription experiments using isolated hepatic nuclei, biotin suppressed the transcription of hepatic PEPCK gene by 55% at 30 min after biotin administration. The inhibition of transcription by biotin is dominant over other stimulatory effects [24].

There are many similarities between biotin and insulin in their action on enzymes of glucose metabolism. Both induce the mRNA that encodes glucokinase, a key glycolytic enzyme, and repress the mRNA that encodes PEPCK, a key gluconeogenic enzyme. This was the first demonstration that a water-soluble vitamin biotin exerted both positive and negative transcriptional control over key enzymes of glucose metabolism.

Intracellular fractionation of biotin in various tissues of the rat and the chicken indicated that a significant amount of biotin was associated with the nuclear fraction [25]. The biotin content of biotin-deficient rat liver is about one tenth that of normal rat liver, and a significant 20% of this is present in the nuclear fraction. Biotin is present in the cell nucleus prepared from various tissues and from cultured cells although the nuclei do not have any carbon-dioxide-fixing capacity, indicating that biotin in the cell nucleus does not function as the prosthetic group of the carboxylases. During biotin deficiency, the biotin in the nuclear fraction seems to be conserved, whereas it is lost preferentially from other cell organelles [26].

11. Mechanism of action of biotin

Biotin in the nucleus is bound noncovalently to a protein. A biotin-binding protein from rat liver nucleus has been isolated [27]. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate provided an apparent molecular weight of 60 kDa. The protein binds to biotin in vitro in a reversible manner, with a B_{max} of 3.54 pmol/μg protein and a K_d for biotin of 2.2×10^{-7} M. It is suggested that this protein might play a role in the expression of the effect of biotin in the cell nucleus.

Current work is directed toward identifying the mechanism of biotin regulation of transcription. Manthey et al. [28] have shown that histones are specific acceptors of biotin transferred from biotinidase, raising the possibility of histones being an endogenous substrate of biotinyl transfer. Biotinylation of histones occurs in vivo in various human cells. There is increased biotinylation in response to cell proliferation [29]. Reduced histone phosphorylation in HCS-deficient patients has been reported. Histones, in vivo, are modified by acetylation, methylation, phosphorylation, ubiquitination and poly(ADP) ribosylation leading to structural changes in the chromatin. By analogy, biotinylation of histones could be another mechanism to alter chromatin structure and, thus, regulate transcription of DNA.

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