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Biotin requirements for DNA damage prevention

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Abstract

Biotin serves as a covalently bound coenzyme in five human carboxylases; biotin is also attached to histones H2A, H3, and H4, although the abundance of biotinylated histones is low. Biotinylation of both carboxylases and histones is catalyzed by holocarboxylase synthetase. Human biotin requirements are unknown. Recommendations for adequate intake of biotin are based on the typical intake of biotin in an apparently healthy population, which is only a crude estimate of the true intake due to analytical problems. Importantly, intake recommendations do not take into account possible effects of biotin deficiency on impairing genome stability. Recent studies suggest that biotin deficiency causes de-repression of long terminal repeats, thereby causing genome instability. While it was originally proposed that these effects are caused by loss of biotinylated histones, more recent evidence suggests a more immediate role of holocarboxylase synthetase in forming multiprotein complexes in chromatin that are important for gene repression. Holocarboxylase synthetase appears to interact physically with the methyl-CpG-binding domain protein 2 and, perhaps, histone methyl transferases, thereby creating epigenetic synergies between biotinylation and methylation events. These observations might offer a mechanistic explanation for some of the birth defects seen in biotin-deficient animal models.

Keywords

biotin; epigenetics; genome stability; human; requirements

1. Introduction

Biotin plays a pivotal role in essential metabolic pathways and epigenetic phenomena in humans. In intermediary metabolism, holocarboxylase synthetase (HLCS) catalyzes the covalent binding of biotin to carboxylases [1–3]. Biotinylated carboxylases are key enzymes in the metabolism of glucose, fatty acids, and leucine [4]. Acetyl-CoA carboxylases 1 and 2 catalyze key reactions in fatty acid synthesis and the inhibition of mitochondrial fatty acid uptake, respectively; 3-methylcrotonyl-CoA carboxylase catalyzes an essential step in leucine metabolism; propionyl-CoA carboxylase catalyzes a key reaction in the metabolism of odd-chain fatty acids; and pyruvate carboxylase is a key enzyme in gluconeogenesis.

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8. Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Biotinidase releases covalently bound biotin from denatured carboxylases to recycle biotin for the synthesis of new carboxylases [5].

In epigenetic pathways, HLCS catalyzes the covalent binding of biotin to histones H1, H3, H4 and, to a lesser extent, H2A [6–11]. Biotinylated histones play roles in the transcriptional repression of genes and repeat sequences [12,13]. Our observation that biotinylation is a natural histone modification was recently confirmed by three independent laboratories [14–16]. These studies included analysis of histone biotinylation by mass spectrometry and suggest that, at least in *Candida albicans*, up to 50% of histones might be biotinylated [15]. In contrast, histone biotinylation is a comparably rare event in humans (<0.1% of histones are biotinylated) [6,16], but the abundance of an epigenetic mark is no marker for its importance. For example, serine-14 phosphorylation in histone H2B and histone poly(ADP-ribosylation) are detectable only after induction of apoptosis and major DNA damage, respectively, but the role of these epigenetic marks in cell death is unambiguous [17,18]. The abundance of histone biotinylation marks is much greater in confined genomic loci compared with bulk histones. For example, about one out of three molecules of histone H4 is biotinylated at lysine-12 (K12) in telomeric chromatin [19].

2. Biotin requirements in humans

The Food and Nutrition Board acknowledges that biotin requirements are unknown [20]. Consequently, no Recommended Dietary Allowances but only recommendations for Adequate Intake are available for biotin in the U.S. Recommendations for Adequate Intake are based solely on the intake of biotin in the general, apparently healthy, population [20]. This approach is flawed in the case of biotin where dietary intake data are only crude estimates. Currently, no studies are available that quantified biotin in foods by using chemically specific assays [21], and it is not clear whether intake estimates exceed or underestimate the true biotin intake. Also, the “normal state” is defined by using biotin-dependent enzymes or urinary biotin metabolites as markers, while ignoring subtle changes occurring at the chromatin level. The uncertainty associated with this approach becomes even more evident when comparing the intake recommendations from 1989 (Adequate Intake = up to 100 µg/d) with those from 1998 (Adequate Intake = 30 µg/d); both recommendations were released by the Food and Nutrition Board at the National Research Council [20,22]. The majority of the over-the-counter biotin supplements in the U.S. contain 300–600 µg of biotin.

3. HLCS and its role in mediating epigenetic synergies between biotin and methyl donors

Consistent with the important roles of HLCS in intermediary metabolism and epigenetics, no living HLCS null individual has ever been reported, suggesting embryonic lethality. HLCS knockdown studies (~30% residual activity) produces phenotypes such as decreased life span and decreased heat resistance in *Drosophila melanogaster* [23] and aberrant gene regulation in human cell lines [13,24,25]. Mutations have been identified and characterized in the human *HLCS* gene; these mutations cause a substantial decrease in HLCS activity and metabolic abnormalities [26,27]. Unless diagnosed and treated early, homozygous severe holocarboxylase synthetase deficiency is characteristically fatal [28]. Three independent cancer and patent databases correlate HLCS loss or mutation with human tumors [29–31].

Some of the effects of HLCS in epigenetic pathways might be mediated by physical interactions of HLCS with other chromatin proteins rather than by HLCS-dependent biotinylation of histones. For example, we have demonstrated that HLCS physically interacts with histone H3 [11] and we have generated evidence that HLCS interacts with the

methylated cytosine binding protein MeCP2 and the histone H3 K9-methyl transferase EHMT-1 {[32]; Y. Li and J. Zempleni, unpublished}. As of today, interactions between HLCS and MeCP2 have been confirmed by using co-immunoprecipitation assays and co-immunoprecipitations [32]. We propose that HLCS is an integral part of a gene repression complex that may also include histone deacetylases and the nuclear co-repressor N-CoR.

4. Biotin deficiency impairs repression of long terminal repeats (LTRs)

Our research revealed mechanistic links among histone biotinylation, repression of recombination hotspots such as LTRs, and genome stability [13,24]. Biotinylation of histones is a gene repression mark, and biotinylation marks are enriched in pericentromeric alpha satellite repeats, telomeres, and LTRs [12,13,19,24,25]. The frequency of retrotransposition events and the number of chromosomal abnormalities increase when LTRs are de-repressed both by biotin depletion and by HLCS knockdown in cell cultures, humans, and *Drosophila* [24]. Atomic force microscopy studies suggest that repression of transcriptional activity by histone biotinylation is caused, at least partly, by chromatin condensation [33].

The majority of mammalian LTRs contain the 13-bp consensus motif located in hotspots for meiotic recombinations [34]. We are currently testing whether de-repression of LTRs provides a mechanistic link between biotin deficiency and aberrant meiosis in humans and animals. Abnormal progression of meiosis would offer a mechanistic explanation for birth defects seen in biotin-deficient animal models [35–38]. It would be of great importance to conduct biotin titration experiments to quantify the levels of biotin needed to prevent genome abnormalities in both somatic and germline cells. These studies are currently underway in our laboratory.

5. Biotin and DNA strand breaks

Evidence suggests that biotin plays a role in causing DNA strand breaks and the cellular response to strand breaks. First, biotin supplementation causes an increase in the expression of the cytochrome P450 1B1 gene in human lymphoblastoma Jurkat cells compared with biotin-depleted cells [39]. The increase in 1B1 expression is associated with an increase in DNA breaks, as judged by comet assay. Please note that this study was conducted in cell cultures and that it is unknown whether these observations have relevance for whole organisms. In this context, it is important to note that low intake of biotin in combination with low intake of multiple other nutrients was associated with increased genome stability in a survey conducted in South Australia [40]. Future studies will need to integrate the observations made in the context of de-repression of LTRs in biotin-deficient cells with those of increased expression of cytochrome P450 1B1 and DNA damage in biotin-supplemented cell cultures. We contend that it is at least theoretically possible that the latter gains relevance in environments.

6. Future directions

Evidence is emerging that HLCS plays a crucial role both in epigenetics and in intermediary metabolism. Therefore studies of HLCS are equally important to studies of biotin. For HLCS, it is important (i) to create a mammalian knockout model, (ii) to resolve its 3D structure by X-ray crystallography; (iii) to identify its binding partners in chromatin; and (iv) to identify single nucleotide polymorphisms that alter catalysis and biotin metabolism. For biotin, it is important (i) to create a reliable database quantifying its contents in foods, and (ii) to obtain reliable estimates of requirements.

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Abbreviations

EHMT-1	euchromatic histone methyltransferase
HLCS	holocarboxylase synthetase
LTR	long terminal repeat
MeCP2	methyl-CpG-binding domain protein 2

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Highlights

- Biotin is covalently attached to both carboxylases and histones.
- Biotinylation of carboxylases and histones is catalyzed by holocarboxylase synthetase.
- Human biotin requirements are unknown.
- Holocarboxylase synthetase or biotinylated histones play a role in the repression of LTRs, thereby maintaining genome stability.
- Holocarboxylase synthetase mediates epigenetic synergies between biotinylation and methylation events.