Utilization of Biotin in Proliferating Human Lymphocytes

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ABSTRACT Lymphocytes are part of the immune system and respond to antigenic stimulation with proliferation. We sought to determine whether mitogen-stimulated, proliferating lymphocytes increase the cellular uptake of biotin and, if so, to identify mechanisms that mediate the increase. Lymphocytes were isolated from human peripheral blood; proliferation of lymphocytes was induced by incubation with pokeweed lectin, concanavalin A or phytohemagglutinin. Biotin uptake was quantitated by determination of [3H] uptake into the lymphocytes during incubation with [3H]biotin after establishing that [3H]biotin is not metabolized within the lymphocytes during the incubation period (<5%). Biotin uptake into proliferating lymphocytes increased to 278–722% of the control values for nonproliferating lymphocytes. Kinetic analysis of biotin transport provided evidence that the increase is mediated by an increased number of transporters on the cell surface rather than by an increase in transporter affinity. Cycloheximide, an inhibitor of protein synthesis, completely suppressed the mitogen-stimulated increase in biotin transport. This observation is consistent with the hypothesis that proliferating lymphocytes increase biotin uptake by increasing the synthesis of new transporters. Biotin affinity and structural specificity were similar in proliferating and nonproliferating lymphocytes, suggesting that mitogens induced an increase in the number of the same transporter molecule that mediates transport in unstimulated lymphocytes. Mitogen-stimulated lymphocytes exhibited 2.5 times greater activities of biotin-dependent β-methylcrotonyl-CoA carboxylase compared with time 0 (at 72 h after addition of mitogen). This observation is consistent with the hypothesis that proliferating lymphocytes increase biotin uptake at least in part to provide adequate coenzyme for biotin-dependent carboxylases. J. Nutr. 130: 335S–337S, 2000.

KEY WORDS: • biotin • carboxylases • humans • lymphocytes • transport

Metabolism of macronutrients in proliferating lymphocytes

Lymphocytes are part of the immune system and respond to antigenic stimulation with proliferation. Proliferation affects the rates of macronutrient uptake and metabolism in lymphocytes. For example, the uptake of alanine, proline and leucine (Segel and Lichtman 1981), and the rate of catabolism of glucose (Loos and Roos 1973, Roos et al. 1972, Roos and Loos 1973) increase in response to mitogenic stimulation. These findings are consistent with the hypothesis that macronutrient demand varies during the cell cycle.

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Proliferation was stimulated by incubation with mitogens for designated periods of time, i.e., pokeweed lectin (0.5–2.0 µg/mL), concanavalin A (5–20 µg/mL) or phytohemagglutinin (5–20 µg/mL) (Zempleni and Mock 1999). Proliferation was confirmed by determination of cellular [3H]thymidine uptake (Zempleni and Mock 1999); lymphocytes that had been stimulated with mitogens for 3 d accumulated at least 20 times more [3H]thymidine than unstimulated controls (P < 0.01). This stimulating index is similar to that noted by Stites (1987).

Then, biotin uptake was quantitated by determination of [3H] uptake into the lymphocytes during incubation with [3H]biotin after establishing that [3H]biotin is not metabolized within the lymphocytes during the incubation period (<5%) and that [3H]biotin does not adhere nonspecifically to lymphocytes (Zempleni and Mock 1998).

Initially, we sought to determine whether proliferating lymphocytes exhibit changes in the transport rate of biotin (Zempleni and Mock 1999). We induced proliferation by incubation with pokeweed lectin, concanavalin A or phytohemagglutinin for intervals that varied between 24 and 168 h. Transport rates of [3H]biotin were determined in both stimulated lymphocytes and unstimulated controls. When the medium contained 475 pmol/L [3H]biotin (a physiologic concentration), nonproliferating controls accumulated 406 ± 201 amol [3H]biotin/(10⁶ cells·30 min). In lymphocytes that had been stimulated with mitogens for 72 h, biotin uptake increased to 278–722% of the control values for nonproliferating lymphocytes. The percentage of increase of [3H]biotin uptake was similar among the three mitogens tested. Activation of biotin transport was maximal at 48–72 h (Zempleni and Mock 1999).

The increased biotin uptake into proliferating lymphocytes was not associated with a global up-regulation of transport activities. This conclusion is based on the observation that uptake rates of urea were similar in mitogen-stimulated, proliferating lymphocytes and unstimulated controls (Zempleni and Mock 1999).

Next we sought to characterize the mechanisms that mediate the increase in biotin uptake (Zempleni and Mock 1999). We hypothesized that the increase was caused either by an increased number of one or more types of biotin transporters per cell or by an increase in the biotin affinity of one or more biotin transporters (or both). Kinetic studies were performed in lymphocytes induced by pokeweed lectin for 3 d; controls were not exposed to pokeweed. Biotin uptake was measured at several distinct biotin concentrations varying from 238 to 2850 pmol/L. Lineweaver-Burk plots constructed from these data revealed intersection near the x-axis for the regression lines of proliferating and nonproliferating lymphocytes; this observation provides evidence that the increase in biotin uptake is mediated by an increase in the number of biotin transporters per cell rather than by an increase in transporter affinity for biotin. The kinetic parameters derived from the Lineweaver-Burk plot are consistent with this interpretation. The maximal transport rates (Vₘₐₓ) of the biotin transporter in proliferating lymphocytes and in controls were 9.1 ± 6.6 and 2.3 ± 1.6 fmol/(10⁶ cells·30 min), respectively (P < 0.05). The Michaelis constants (Kᵥ) were 2.4 ± 1.7 and 3.7 ± 3.4 nmol/L, respectively, in proliferating lymphocytes and in controls (P = 0.86, not significant).

Moreover, mitogenic stimulation of biotin transport was inhibited completely by the addition of cycloheximide, an inhibitor of protein synthesis, to the medium (Zempleni and Mock 1999). This observation is consistent with the hypothesis that proliferating lymphocytes increase biotin uptake by increasing synthesis of the biotin transporter.

Theoretically, the increase in biotin uptake might have been mediated by increased synthesis of a different biotin transporter than the one responsible for transport in nonproliferating lymphocytes (Zempleni and Mock 1999). Other biotin transporters have been identified in other tissues. For example, rat placenta transports biotin by using a relatively unspecific biotin/lipoic acid/pantothenic acid transporter (Prasad et al. 1998). Potentially, proliferating lymphocytes might synthesize this “multivitamin” transporter at an increased rate rather than the specific biotin transporter. To investigate this possibility, we examined the effect of lipoic acid on biotin transport in proliferating and nonproliferating lymphocytes. Extracellular lipoic acid did not significantly reduce [3H]biotin uptake into proliferating lymphocytes or nonproliferating lymphocytes at either physiologic (500 nmol/L) or pharmacologic (5000 nmol/L) concentrations of lipoic acid. These data provide evidence that the primary transporter of biotin is the same structurally specific transporter in both proliferating and nonproliferating lymphocytes.

Activities of biotin-dependent carboxylases are increased in mitogen-stimulated lymphocytes

We propose that one or more of the following three mechanisms generates an increased demand for biotin in proliferating lymphocytes: 1) Proliferation causes increased biotin uptake to meet demand for coenzyme for biotin-dependent carboxylases, 2) Proliferation causes increased biotin uptake to meet demand for biotin used to biotinylate histones, and 3) Proliferation causes increased biotin uptake to meet demand for biotin used to biotinylate histones (Hymes 1994, Hymes and co-workers have shown that biotinidase (EC 3.5.1.12) catalyzes this biotinylnation and that histones act as specific biotinyl acceptors (Hymes et al. 1995, Hymes and Wolf 1999). Biotinidase is ubiquitous in mammalian cells and 25% of the cellular biotinidase activity is located in the nuclear fraction (Pispa 1965). Biotinylination of histones suggests that biotin might play some role in DNA transcription and replication. In analogy, transcription and replication of DNA are affected by acetylation, methylation phosphorylation or ADP-ribosylation of histones (Ausiello holde 1986, Boulikas 1988, Boulikas et al. 1990). Hohmann 1988, Kaye and Sommerville 1965, Hohmann 1988, Kaye and Sommerville 1965, Lee et al. 1993, Paik and Kim 1969, Roth and Allis 1992, Sommerville et al. 1993). 3) Proliferating lymphocytes increase biotin uptake to sequester plasma biotin from invading microorganisms. By analogy, an antimicrobial effect of biotin withdrawal has been proposed to operate in eggs. Egg white contains the protein avidin (0.05% of total protein), which binds biotin tightly; the dissociation constant of the biotin-avidin complex is 10⁻¹⁰ mol/L (Green 1975). The tight binding of biotin to avidin prevents diffusion of biotin to the microbes, and avidin is resistant to a variety of proteases (thus preventing microbes from releasing biotin from avidin).

We have conducted preliminary studies regarding the effect of lymphocyte proliferation on activities of biotin-dependent β-methylcrotonyl-CoA carboxylase (EC 6.4.1.4) and propionyl-CoA carboxylase (EC 6.4.1.3) (Zempleni, J. and Mock, D.M., unpublished observations). In these studies, we stimulated proliferation of lymphocytes by incubation with either pokeweed lectin (2.0 µg/mL) or concanavalin A (20 µg/mL) for up to 144 h; controls were incubated without mitogens. At timed intervals, aliquots were collected. Activities of β-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase were assayed using a method based on the incorporation of
radiolabeled bicarbonate into the product of the carboxylase reactions (Zempleni et al. 1997).

The activity of β-methylcrotonyl-CoA carboxylase increased with proliferation, reaching a maximal increase 72 h after addition of either mitogen; at that time, β-methylcrotonyl-CoA carboxylase activity was ~2.5 times greater than at time 0, i.e., before addition of mitogens to the medium (P < 0.05). At 144 h, β-methylcrotonyl-CoA carboxylase activity in mitogen-stimulated lymphocytes was not significantly different from time 0 (P = 0.06).

Similarly, the activity of propionyl-CoA carboxylase increased by 10–50% with proliferation; activity reached a maximum at 72 h. The increase did not quite reach statistical significance (P = 0.06).

These pilot studies of carboxylase activities suggest that proliferating lymphocytes increase biotin uptake to provide coenzyme for biotin-dependent carboxylases.

LITERATURE CITED


