S-ADENOSYL-L-METHIONINE DECARBOXYLASE DURING LYMPHOCYTE TRANSFORMATION: DECREASED DEGRADATION IN THE PRESENCE OF A SPECIFIC INHIBITOR*

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SUMMARY: S-Adenosyl-L-methionine decarboxylase (AmDC) activity increased 20- to 25-fold in a biphasic manner during Concanavalin A-induced lymphocyte transformation. When a potent inhibitor of this enzyme, methylglyoxal bis(guanylhydrazone), was added to transforming cultures, AmDC rapidly increased beyond the fully induced level and eventually reached a specific activity 2500 times that found in non-transformed lymphocytes. Measurements of the decline in enzyme activity in the presence of cycloheximide indicated that the half-life increased from 40 minutes to at least 20 hours during inhibitor treatment. It is likely that this change in the rate of AmDC degradation was primarily, if not solely, responsible for the large increases observed.

The initial report of Williams-Ashman and Schenone (1) demonstrated that methylglyoxal bis(guanylhydrazone) (MGBG) is a potent inhibitor of mammalian, putrescine-activated S-adenosyl-L-methionine decarboxylase (AmDC). We were subsequently able to show that MGBG added to lymphocyte cultures inhibited intracellular spermidine and spermine accumulation during transformation induced by Concanavalin A (ConA) (2-4). We report here that the addition of MGBG to transforming lymphocyte cultures caused a striking increase in AmDC levels, while the intracellular enzymatic activity was apparently inhibited. The increase appears to be mediated by changes in the rate of degradation of the enzyme.

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METHODS:

Lymphocytes from bovine lymph nodes were cultured and transformed by Concanavalin A (Miles) at a final concentration of 15 µg/ml (4). Methylglyoxal bis(guanylhydrazone) was obtained from Aldrich Chemical Co.

For preparation of cell-free extracts, cultures containing 3 x 10^8 cells were centrifuged (300g, 10 min.) and washed once with Puck's Saline G (5). The cellular pellet was homogenized in a Potter-Elvehjem homogenizer with a teflon pestle in 0.5 ml of 25 mM Tris-HCl (pH 7.5) containing 0.1 mM disodium EDTA and 5 mM dithiothreitol as described for preparation of ornithine decarboxylase from rat prostate (6). The homogenate was centrifuged (100,000g, 100 min.) and dialyzed twice against 100 volumes of homogenization buffer at pH 7.2. Dialysis effectively removed MGBG as judged by mixing experiments. AmDC activity was measured by release of $^{14}CO_2$ from S-adenosyl-L-methionine [1-^{14}C] (7.7 mCi/mmole, New England Nuclear) at 37°C. The standard incubation mixture was buffered with 0.1 M sodium phosphate (pH 7.4) and contained putrescine (2.5 mM final concentration) and S-adenosyl-L-methionine (0.2 mM final concentration) in a total volume 0.3 ml. The CO_2 evolved was trapped and counted as previously described (7) following the addition of 25 µl of 100% (w/v) trichloroacetic acid. Enzyme assays from control cultures (minus MGBG) were routinely run for 30 minutes with up to 400 µg protein per assay and were linear with respect to protein concentration and time. To maintain this linearity, extracts from MGBG-treated cultures were diluted and the assay time reduced.

RESULTS:

The changes in AmDC activity during ConA-stimulated lymphocyte transformation are shown in Figure 1. Activity was detectable in unstimulated cultures, increased significantly by 3 hours, and reached a level 15 times the initial by 6 hours. Activity remained relatively constant between 6 and 24 hours, after which a second increase was observed to a final specific activity 20 times the initial.

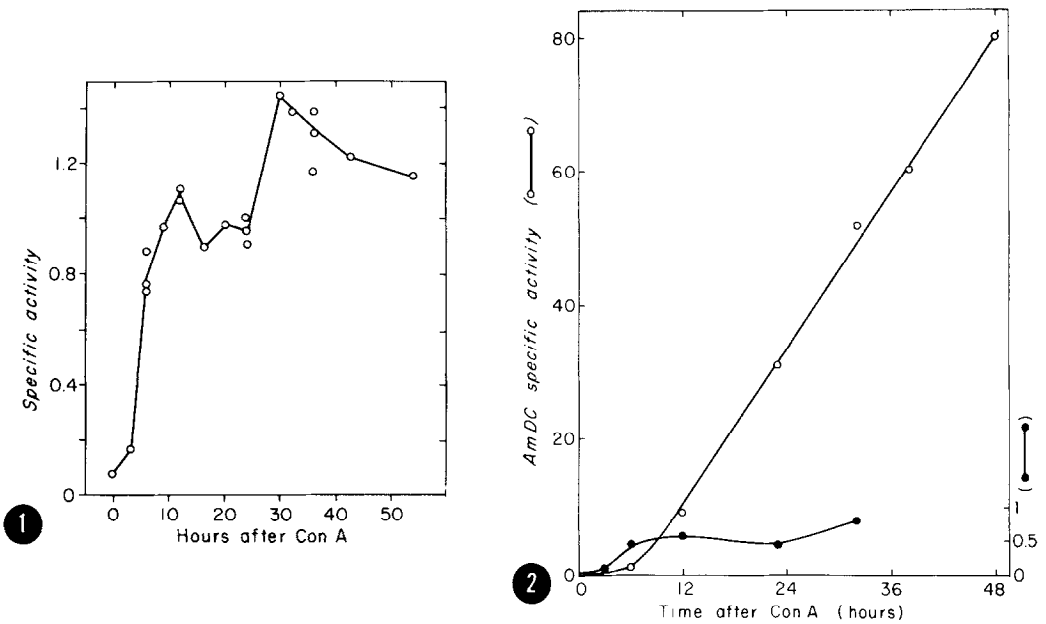

Figure 1. Changes in AmDC activity during ConA-induced lymphocyte transformation. Activity was measured with dialyzed cellular homogenates in the presence of 2.5 mM putrescine. The putrescine-dependent specific activity (nmole/hr x mg protein) is plotted versus time. Each point represents the average of two activity measurements with a single homogenate (maximal difference 5%).

Figure 2. Effect of MGBG on the level of AmDC in transforming lymphocytes. MGBG was added to a final concentration of 20 μM immediately after the addition of ConA at 0 hours. Cultures were harvested and homogenized at the times indicated and the dialyzed homogenates assayed in duplicate in the presence of 2.5 mM putrescine. Control without MGBG (●); MGBG added at 0 hours (○). Note difference in axes.

When MGBG was added to ConA-stimulated cultures, much larger increases in AmDC were observed (Figure 2). MGBG was added to a final concentration of 20 μM at the time of addition of ConA. During the first 6 hours, AmDC increased 2-3 times faster in the presence of MGBG than in control ConA-stimulated cultures (note the difference in scales). After this initial lag, AmDC increased linearly in the presence of MGBG and reached levels 100 times the fully induced control level by 48 hours.[1] If MGBG was added to a final

[1]The specific activity of all the preparations in this particular experiment were approximately 50% that normally observed. The reason for the difference is not understood.

concentration of 40 μM at 24 hours after the addition of ConA, AmDC activity was elevated to a level 20 times the control within 4 hours of MGBG addition. Activity continued to increase linearly for at least 18 hours and the final activity was 100 times the 42 hour control.

The experiment shown in Figure 3 suggests that the MGBG-mediated increase in AmDC was due to stabilization of the enzyme. In this experiment, protein synthesis was blocked (97% inhibition of ^{3}H-leucine incorporation) with cycloheximide and the rate of loss of enzymatic activity was measured. The activity in the 24-hour control culture decayed rather rapidly ($t_{\frac{1}{2}}$ = 43 min.). In the presence of MGBG, AmDC activity was stable over the 90 minute period of the experiment. On the basis of these data, the half-life could be no shorter than 20 hours. This large difference in apparent half-life suggests that the MGBG-induced increase in enzyme activity was mediated primarily by a decrease in the rate of enzyme degradation.

DISCUSSION:

The large, early changes in AmDC activity observed during lymphocyte transformation have not been observed in other systems undergoing a proliferative response (e.g., regenerating liver). The reason may be that AmDC is fairly high in normal liver and apparently is not limiting the rate of spermidine and spermine formation during the first day of regeneration (8,9). Small elevations in specific activity (3-fold) are observed by the second day of regeneration (9). These increases may correlate physiologically with the second phase of the AmDC induction observed during lymphocyte transformation.

MGBG apparently has a high affinity for the lymphocyte enzyme since activity was inhibited by 50% at 0.2 μM (4). At the concentrations of MGBG used in the experiments presented here, the cellular synthesis of spermidine and spermine would be completely blocked (4), and the enzyme saturated with the inhibitor. Under these conditions, the enzyme was

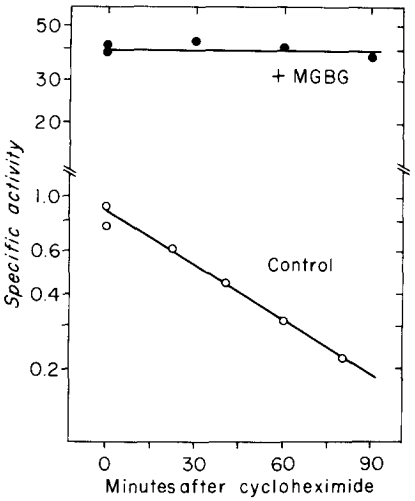

Figure 3. Influence of MGBG on the half-life of AmDC. Lymphocyte cultures were incubated with ConA for 24 hours. Cycloheximide (Sigma) was added to control cultures (25 µg/ml) at 24 hours and the residual activity determined at the times indicated. MGBG (8 µM final) was added to the other cultures at 24 hours and they were incubated until 36 hours. Cycloheximide was then added and the enzymatic activity remaining in each culture determined at the times indicated. Activity was determined in duplicate with dialyzed homogenates in the presence of 2.5 mM putrescine. The semi-logarithmic plot of specific activity (nmole/hr x mg protein) versus time is shown. Control at 24 hours (0); MGBG present 24 to 36 hours (●).

markedly, and perhaps completely, resistant to intracellular degradation. This does not appear to be due to a general effect on protein turnover since ornithine decarboxylase was also elevated (2-3 fold) during MGBG treatment (2), with no change in half-life (unpublished). Taken together these results suggest that MGBG decreases degradation of AmDC by directly interacting with the enzyme. The in vivo stabilization of an enzyme by its specific inhibitor (10) or substrate (11) is not unprecedented. For example, dihydrofolate reductase is stabilized by amethopterin in growing L 1210 cells (10). The difference in turnover rates resulted in increased cellular levels of this enzyme (12).

The large, MGBG-mediated increase in AmDC should be of considerable value to those workers who are attempting to purify and characterize this enzyme and find themselves limited in starting material.

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