REGULATION OF ARGINASE ACTIVITY IN CHANG LIVER CELLS IN THE ABSENCE OF NET PROTEIN SYNTHESIS

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Arginase activity was studied in Chang liver cells, maintained for one day in a growth medium, from which one essential amino acid was omitted. To avoid the complexity of whole animals, cultured cell strains have been used to study the influence of environmental factors on specific enzyme synthesis. In view of the inverse relationship between cellular differentiation and cell multiplication (Agrell, 1964), the regulation of the synthesis of specific proteins under conditions of restricted growth is of particular interest.

In Chang liver cells, maintained in an amino acid-deficient medium, over all protein synthesis decreases rapidly (Eliasson, Bauer and Hultin, 1967). At the same time the in vitro activity of the ribosomes and the proportion of active polysomal aggregates diminish. These effects are readily reversed on the restitution of an adequate environment. The restoration of the polysomal structures and the normal rate of protein synthesis are insensitive to actinomycin D, suggesting that stable messenger RNA is preserved in the starved cells in an inactive state.

From the point of view of enzymatic differentiation, the following question appeared to be of importance: Does the decrease in over all protein synthesis in "starved" cells, involve a decreased rate of translation of all messenger present, or is the translation of some species of messenger prevented, while others are still available for translation?

The following information from earlier experiments concerns the properties and formation of arginase in cell strains of human origin: 1. Arginase activity, as determined in cell lysates, depends on the amount of enzyme present, rather than on the presence of inhibitors or activators (Schimke, 1964; Eliasson, 1965; Eliasson and Strecker, 1966). 2. The continuous destruction of the enzyme during general protein turn-over can be prevented by the addition of manganese ions to the culture medium (Schimke, 1964; Eliasson and Strecker, 1966). 3. The formation of arginase in Chang cells is repressed by a metabolic product of the arginase-initiated reaction sequence from arginine to proline (Eliasson and Strecker, 1966). An increased arginase activity can thus be produced, by increasing the concentration in the culture medium of the amino acids lysine, leucine, or valine, which are known to inhibit enzymes in the reaction chain from arginine to proline. An increase in arginase activity can also be brought about by decreasing the substrate level in the same reaction sequence. (This can be achieved by making the availability of arginine growth limiting.) Finally, a decrease in arginase activity takes place in Chang cells grown in the presence of proline, the product of the same reaction sequence. 4. When Chang cells, after preincubation for one day in a deficient medium, are again transferred to a normal growth medium, an extremely rapid increase in arginase activity takes place during the first 4-6 hours after the restitution of the normal cell environment (Eliasson, 1967). Since this increase in enzymatic activity can be stopped by the addition of puromycin but is not prevented by the addition of actinomycin D, enzyme formation seems to occur in response to pre-formed messenger RNA. This messenger RNA has a minimal life time of 4 hours (it has not been possible to determine the maximal life time of the messenger).

MATERIAL AND METHODS

Chang liver cells were grown in suspension cultures in a slightly

modified Eagle's minimal medium, containing 10% horse serum as previously described (Eliasson and Strecker, 1966). Single experiments were started with cells in a state of rapid growth, with a generation time of 30 to 35 hours. Cells were sedimented and transferred to a medium modified as described in the different experiments. Since arginase activity in cells from different stock cultures showed considerable variations (Eliasson and Strecker, 1966), experimental studies always were conducted in a parallel manner with cells derived from the same stock culture.

Arginase and protein determinations were carried out as previously described (Eliasson and Strecker, 1966). All determinations were made in duplicate. Duplicate enzyme determinations agreed within 10%. Specific arginase activity is expressed as μmoles of urea formed per hour and mg protein.

RESULTS AND DISCUSSION

The over all protein synthesis of Chang cells maintained in a glutamine- or arginine-free medium rapidly decreases to a rate corresponding to 20-30% of the rate of synthesis in exponentially growing cells (Eliasson, Bauer and Hultin, 1967). This rate seemed to balance the continuous destruction of cellular proteins, since the over all protein content of the cultures did not change significantly during one day in the deficient medium.

Product repression of arginase synthesis in non-growing cells. Arginase activity increased only slightly over a period of one day in a glutamine deficient medium, containing 0.1 mM $MnCl_2$ as a stabilizer of the enzyme (Fig. 1). A more than 100% increase in arginase activity could be produced by increasing the concentration of lysine (a competent inhibitor of arginase) from 0.4 mM to 6 mM in the growth medium (Fig. 1A). A similar increase in arginase occurred, when arginine instead of glutamine was omitted from the culture medium, during the

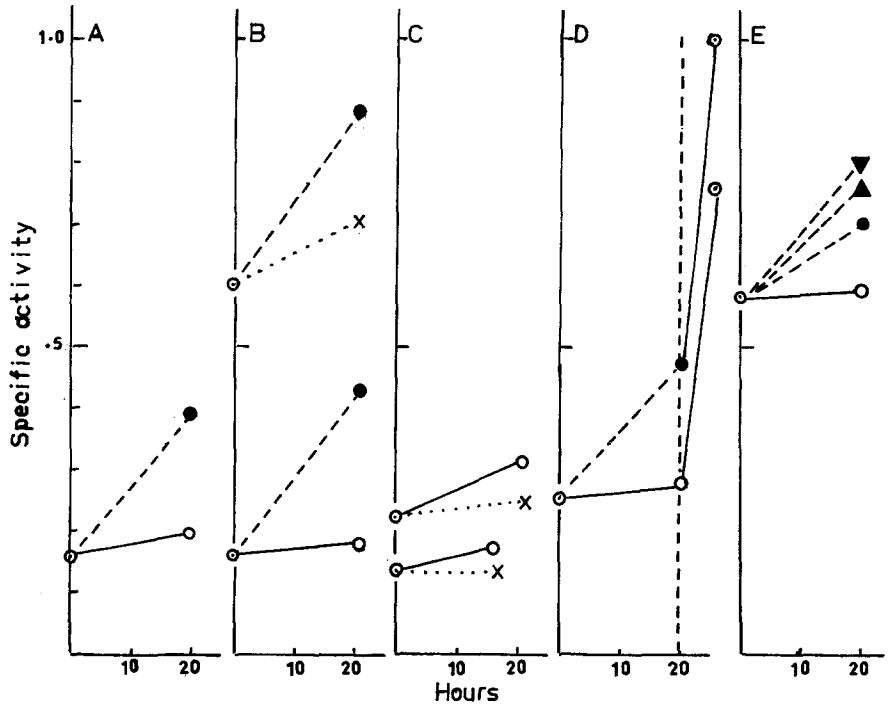

Fig. 1. Change in specific arginase activity in Chang cells during maintenance in amino acid-deficient media.
(0.1 mM $MnCl_2$ present as an enzyme stabilizer).
No glutamine (o———o).
A. No glutamine, 6 mM lysine added (-----•).
B. No arginine (-----•). No arginine, 6 mM proline added (....x).
C. No glutamine, 6 mM proline added (....x).
D. No glutamine, 6 mM leucine, 6 mM arginine (-----•), at 20 hours cells were transferred to a normal growth medium without other additions than 0.1 mM $MnCl_2$ (———o).
E. No glutamine, actinomycin D added in the following concentrations: 0.1 µg/ml (-----▼), 0.05 µg/ml (-----▲), 0.025 µg/ml (-----•).

starvation period (Fig. 1B). The increase in arginase activity during glutamine or arginine starvation was effectively prevented by addition of proline at a 6 mM concentration (Fig. 1B, 1C, 2).

These results suggest that arginase formation in absence of net protein synthesis is regulated in the same way as in normally growing cells - by product repression.

Repression of arginase synthesis on a level separate from that at which formation of specific arginase messenger RNA takes place. When cells from a glutamine-deficient medium containing 6 mM proline were transferred to a normal growth medium (without proline), an immediate increase in arginase activity occurred (Fig. 2). The increase in enzymatic activity was not prevented by the addition of actinomycin D at a

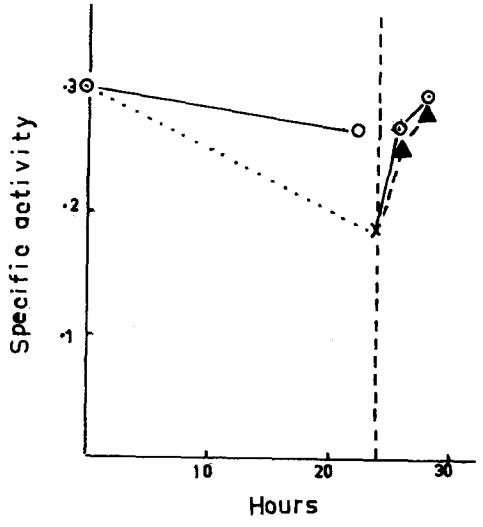

Fig. 2. Specific arginase activity in cells incubated for 24 hours in a glutamine-free medium ⊙———o and in the same medium containing 6 mM proline (.....x). At 24 hours cells starved in presence of proline, were transferred to a normal growth medium without proline (⊙———⊙) or to the same medium with 0.5 µg/ml of actinomycin D added (▲-----▲). The experiment was performed in the absence of manganese. A continuous destruction of arginase at a rate of 2-3% per hour must therefore be taken to account, when comparing this experiment with the experiment in Fig. 1C (Eliasson, 1967).

concentration of 0.5 µg/ml. This concentration has been shown to be sufficient to decrease the rate of incorporation of C^{14} uridine into RNA in prestarved Chang cells to < 5% of the incorporation activity of normally growing cells (Eliasson, Bauer and Hultin, 1967). The result of this experiment, which is in accordance with other similar experiments, suggests that arginase messenger RNA is still present in the cells after one day of glutamine starvation in presence of proline.

Therefore, inhibition of arginase synthesis during the starvation period seems not to be due to an absence of specific messenger RNA.

A similar conclusion may be drawn from the experiment shown in Fig. 1D, i.e. that the rate of enzyme formation is not immediately related to the amount of messenger present. In this experiment cells were maintained for one day in a glutamine-free medium containing leucine and arginine* at 6 mM concentrations. A 100% increase in arginase activity occurred during the starvation period. When the cells were subsequently transferred to a normal growth medium, the enzymatic activity showed an actinomycin-insensitive increase of the same rate as that in cells which had been maintained in a glutamine-deficient medium containing normal concentrations of leucine (0.4 mM) and arginine (0.6 mM).

A regulation of arginase synthesis on a level separate from the formation of messenger RNA also was indicated by the experiment shown in Fig. 1E. Arginase formation during glutamine starvation was stimulated in presence of actinomycin D at concentrations of 0.025-0.1 µg/ml. These concentrations have been shown to cause a 70 to 90% inhibition of the incorporation of C^{14} uridine into RNA in glutamine starved cells (Eliasson, 1967).

The present results suggest that a stable messenger RNA for arginase synthesis is present in non-growing Chang cells, and that this messenger might be either in an active state or in an inactive state depending on the intracellular concentration of a metabolic repressor. Furthermore, the stimulation of arginase synthesis in non-growing cells by actinomycin D indicates that the transformation of the messenger into the inactive state might involve an actinomycin sensitive mechanism.

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* Preliminary experiments showed that the increase in arginase activity in the presence of increased concentrations of leucine or valine is enhanced by a simultaneous increase in the concentration of arginine in the growth medium. (An increased concentration of arginine alone has very little effect on arginase activity in normally growing Chang cells.)

Mechanisms selectively regulating protein synthesis at the translational level may evidently be of importance for the development and maintenance of the enzyme pattern in cells containing stable messenger RNA. The possibility of a regulation of protein synthesis at the translational level in differentiating animal cells has been discussed recently (Spirin, 1966).

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