Arginase Activity during the Growth Cycle of Chang’s Liver Cells*

EVA E. ELIASON AND HAROLD J. STRECKER†
From the Wenner-Gren Institute for Experimental Biology, University of Stockholm, Stockholm, Sweden

(Received for publication, July 19, 1966)

SUMMARY

An increase of arginase activity has been produced in Chang’s liver cells in suspension cultures by increasing the concentration in the growth medium of lysine, leucine, valine, or ornithine. These amino acids are known to inhibit enzymes in the arginase-initiated reaction sequence from arginine to proline. An increase in arginase also occurred with o-aminobenzaldehyde, a compound which reacts with a metabolite (Δ²-pyrrroline 5-carboxylate) of the same reaction chain. A stimulation of arginase has been also shown following substitution of citrulline for arginine in the growth medium. A decrease in arginase was found after addition of proline, a product of the same reaction sequence.

Experiments in which protein synthesis was inhibited by puromycin indicate that the rate of accumulation of enzyme protein was involved rather than an activation or inactivation of preformed enzyme.

When arginase was stabilized by the addition of manganese to the growth medium, the compounds mentioned above produced the same results as before, although greater in magnitude, indicating an effect on the rate of synthesis rather than on the rate of degradation of the enzyme during general protein turnover.

These results suggest that synthesis of arginase in Chang’s liver cells during a normal 4-day growth cycle is regulated, in part at least, by product repression.

Arginase activity related to dietary and hormonal influences has been studied extensively in livers from ureotelic animals (1–13). With few exceptions (9, 12), changes in liver arginase are coordinated with changes in other enzymes of the urea cycle. Arginase, however, in contrast to carbamylphosphate synthetase and ornithine transcarbamylase, is widely distributed, and is present in many tissues and cells in which the complete urea cycle has not been observed.

All established animal cell strains in tissue culture, including cell strains derived from mammalian liver, appear to lack ornithine transcarbamylase (14–18). It seems likely in such cells that the reaction sequences from arginine to proline or to glutamic acid via Δ²-pyrrroline 5-carboxylate (19–22) could constitute a major route for the metabolism of ornithine produced by the action of arginase. The enzymes catalyzing the individual steps of this reaction sequence have been shown to be present in Chang’s liver cells (23).

Several investigations have shown variations in arginase activity in animal cells in tissue culture related to changes in the nutrient medium. Schimke has shown that manganese in the medium brings about greater arginase activity in HeLa S2 cells (18). Increases in arginase in cultured cells related to the concentration of arginine (and citrulline) in the nutrient medium have also been reported (24–29). Earlier findings in this laboratory that glucose decreased arginase activity of Chang’s liver cells cultivated in continuous flow cultures (29) suggest that this enzyme might be regulated by a mechanism similar to that of catabolite repression in microorganisms. Since catabolite repression of individual enzymes in microorganisms may be related to the formation of a metabolic product of the reaction sequence in which the enzyme is active (30–32), it seemed reasonable to investigate whether variations in intracellular concentration of the products of the metabolic sequence, mutated by arginase, affected the level of arginase activity in Chang’s liver cells. These studies were conducted during a normal 4 day growth cycle in order to compare the results with the parallel study of ornithine δ-transaminase (23).

METHODS

Chang’s liver cells, obtained from Microbiological Associates, Bethesda, Maryland, were cultivated in suspension in a slightly modified Eagle medium as described previously (23). The stock cultures were propagated through serial dilution twice a week. When not specifically indicated in the text, single experiments were begun with cells from a stock culture which had been diluted 4 days earlier. The cells were sedimented and experiments were started in fresh medium. Cell counting and testing for viability and pleuropneumonia-like organism contamination were conducted as previously described (23).

1 H. J. Strecker, unpublished data.
Arginase in Chang's Liver Cells

Vol. 241, No. 24

Fig. 1. Effect of Mn++ on specific activity of arginase (A) and on multiplication of Chang's liver cells in suspension culture during a 4-day growth cycle. With Mn++: •—•; without Mn++, ○—○. The units on the ordinate of B are at a cell density of 10^6 cells per ml.

with arginine-free medium, the serum used was passed through Sephadex G-50 (33).

Enzyme Assays—The cells were collected by centrifugation at 300 × g for 7 min, washed with cold Hanks' salt solution (34), and resuspended in 0.05 M Tris buffer, pH 7.8, at a cell density of 10^6 cells per ml. The cell suspensions were kept frozen at −15°. Arginase activity was determined in the cell suspensions after thawing according to Schimke (27). Protein concentration was determined by the method of Lowry et al. (35), and specific enzymatic activity is expressed as micromoles of urea formed per hour per mg of cell protein. Reagent solutions were prepared in glass-distilled water. All reagents were of the highest purity commercially available.

RESULTS

Arginase activity in Chang's liver cells showed considerable variability depending on culture conditions. To obviate uncontrolled variations during the experimental period, the methods of cell culture both for the experiments and for the maintenance of stock cultures were standardized carefully. In spite of these measures, some variability of the arginase activity of the control cultures was encountered from experiment to experiment, perhaps because of undetermined differences in the serum employed (cf. Reference 36). The control and experimental studies were therefore routinely conducted in a parallel manner with cells derived from the same stock culture and with serum from the same batch.

During a 4-day growth cycle, following transplantation of the cells from a stock culture into fresh medium without added manganese as described above, only a slight increase in specific arginase activity was observed. However, with added manganese, a considerable rise in arginase was seen during the same period of time (Fig. 1A). This effect has been shown also with HeLa cells (18). The data on cell growth are presented in Fig. 1B. The increase in arginase activity in response to manganese ceased immediately upon the addition of puromycin. This result, together with the fact that arginase determinations were made after preliminary activation of the enzyme by manganous ions at 30 mM concentration, indicates that the effect of manganese in the growth medium was related to an accumulation of enzyme protein in the cells rather than to an activation of inactive enzyme already present. Since protein turnover seems to be a continuous process in animal cells (37, 38), the accumulation of enzyme could depend on either increased synthesis or decreased destruction or inactivation of the enzyme. Schimke already has shown that manganese prevents loss of arginase in HeLa cells (18). The following experiment indicated that this was true also for Chang’s liver cells. Logarithmically growing Chang’s liver cells were transplanted into Eagle medium lacking the essential amino acid, glutamine. Cell multiplication and net increase in protein stopped while arginase activity decreased, indicating a destruction or inactivation of the enzyme. This decrease in arginase occurred at about the same rate irrespective of puromycin addition (7 µg per ml), indicating that very little synthesis of this enzyme took place in the glutamine-starved cells. If manganese was added to the starvation medium, no decrease in arginase activity took place. Puromycin in addition to manganese had no additional effect (Fig. 2).

It thus seemed evident that arginase was stabilized by manganese in nongrowing cells. If the same is true also for growing cells, the increase in arginase activity obtained with manganese ions as shown in Fig. 1 represents the synthesis of enzyme, while the approximately stable level in the control experiment lacking manganese might be interpreted as the result of a simultaneous synthesis and degradation of the enzyme. The stabilizing effect of manganese on arginase seemed to offer an opportunity to study the influence of substances which conceivably could affect the level of metabolic products of arginine and thus perhaps bring about some changes in arginase activity. Some of these compounds have been shown also to alter the level of ornithine δ-transaminase activity in Chang’s liver cells (23). The experiments described below were therefore performed in the presence of Mn++ and of puromycin on the specific activity of arginase during incubation in glutamine-free medium.

Fig. 2. Effects of Mn++ and of puromycin on the specific activity of arginase during incubation in glutamine-free medium. Without Mn++ or puromycin, O——O; with Mn++ only, •——•; with puromycin only, O——O; with both Mn++ and puromycin, - - - - - .
as well as in the absence of manganese. In the presence of 0.1 mM MnCl₂, cell growth was inhibited by 20 to 50% (Fig. 1B).

Effect of Lysine, Leucine, and Valine—The following amino acids at 6 mM concentration were added separately to the medium and arginase activity in the Chang’s cells determined at intervals during a 3- or 4-day growth cycle: ornithine, glycine, lysine, leucine, valine, glutamic acid, and proline. Of these, only lysine, leucine, or valine caused an increase in activity, observable either with or without manganese in the growth medium (Fig. 3, A and B). Leucine or valine had no effect on the growth and viability of the cells, but lysine at 6 mM concentration completely inhibited cell multiplication in the absence of manganese and inhibited growth about 50%, as compared with the control growth, in the presence of 0.1 mM MnCl₂. The increase in enzymatic activity was immediately stopped by the addition of puromycin (10 μg per ml), indicating that protein synthesis indeed was involved in the accumulation of enzyme (Fig. 4).

The enhancement of arginase activity obtained with lysine, leucine, or valine even in the presence of an optimal manganese concentration indicates that their mode of action is not merely stabilization of the enzyme.

These three amino acids are effective inhibitors of enzymes in the metabolic sequence from arginine to proline. All inhibit arginase (39); leucine and valine inhibit ornithine 6-transaminase as well (40). It seems possible that relatively high concentrations of these amino acids decrease the rate of formation of a product of arginine metabolism that otherwise would accumulate in the cells and function to repress arginase synthesis. Lowering the concentration of this hypothetical metabolic repressor could account for the stimulation of enzyme synthesis. Support for this hypothesis was obtained also from experiments with o-aminobenzaldehyde.

o-Aminobenzaldehyde—This has been used to trap Δ¹-pyrroline 5-carboxylate, the immediate product of the ornithine δ-transaminase reaction (19). At a concentration in the medium of 300 μg per ml, o-aminobenzaldehyde strongly stimulated arginase activity, both in the presence and absence of manganese (Fig. 5, A and B). Interpretation of the effect of o-aminobenzaldehyde is complicated because of its growth-inhibiting effect (Fig. 5C), but, since not only the specific activity but also the total activity of arginase in the whole culture was higher after cultivation with added o-aminobenzaldehyde, there seems to be little doubt that the compound caused an accumulation of arginase.

A series of experiments was undertaken with the purpose of increasing the amount in the culture of a hypothetical end product repressor for arginase.

Δ¹-Pyrroline 5-Carboxylate—When added to the medium at 6 mM concentration, Δ¹-pyrroline 5-carboxylate had no effect on the arginase activity of the cells. The lack of effect of pyrroline 5-carboxylate might be due, however, to the instability and relative impurity of the compound (41).

Glutamic Acid—Added at a concentration of 6 mM, both with and without manganese, glutamic acid was also without effect on the development of arginase activity.

Proline—This acid, on the other hand, slightly repressed the development of arginase activity in the absence of manganese. In the presence of 0.1 mM MnCl₂, the repression by proline was more clearly evident, so that the normal increase in arginase was not observed (Fig. 6A). In addition, proline effectively inhibited the increase of arginase caused by leucine (plus manganese) (Fig. 6B). Since it seems improbable that proline counteracts the stabilization of the enzyme by manganese, and since proline had no appreciable effect on the growth rate of the cells, it is conceivable that proline either directly or indirectly causes the accumulation of a repressor of enzyme synthesis. Since proline, in the absence of manganese, only weakly repressed arginase activity during 70 hours, the effect of proline on cells grown for a longer period of time was studied. Two cultures
Arginase in Chang's Liver Cells

Vol. 241, No. 24

FIG. 5. A, specific activity of arginase in Chang's liver cells during a 4-day growth cycle in normal medium containing o-aminobenzaldehyde (●●●) and control experiment (○○○). B, the experiments and conditions are the same as A except that 0.1 mM Mn++ was added to the growth medium (●●●●●) with o-aminobenzaldehyde, control experiment, ○○○○○. C, Effect of o-aminobenzaldehyde on multiplication of cells in normal complete medium containing also 0.1 mM Mn++ (○○○○○); control experiment without o-aminobenzaldehyde (●●●●●). All other conditions are the same as before.

were propagated by serial dilution every 2nd day, one in ordinary medium and the other in medium containing 6 mM proline. The cell growth was approximately the same in the two cultures (Fig. 7B). Fig. 7A shows that under these conditions proline caused the specific activity of arginase to decrease from 0.6 to 0.1, while the control fluctuated in activity in the range of 0.6 to 1.0. The effect of proline on arginase activity is in good agreement with Klein's observation (24) that arginase could be induced in freshly isolated cells grown in an Eagle medium but not in a medium (Parker 199) that contained proline (42). Eagle has found that growth in the presence of proline had no effect on the ability of cultured human cells to synthesize proline from arginine (43). This result, however, need not be in disagreement with the observed effect of proline on arginase, because arginase, under these experimental conditions, might not be the rate-limiting enzyme of the pathway.

Substitution of Citrulline for Arginase—Another way of decreasing formation of a product of a sequence of reactions is to decrease the concentration of substrate for a rate-limiting step in a reaction chain. The enzymes synthesizing arginine from citrulline in cultured animal cells have been shown to be of low activity relative to arginase (18, 26, 27). If this same relationship held in Chang's liver cells, substituting citrulline for arginine in the growth medium might limit the concentration of arginine sufficiently to make the arginase step rate-limiting in the over-all sequence and to decrease the rate of production of the metabolic end product. The cells were therefore transferred to an arginine-free medium with 0.6 mM citrulline instead of arginine. Cell growth was inhibited by 30% in the citrulline-containing medium, indicating that the availability of arginine for protein synthesis indeed might be growth-limiting. Fig. 8 shows the stimulating effect of the citrulline-containing, arginine-less medium on arginase activity, with manganese present. (Growth-limiting amounts of arginine in the absence of citrulline also stimulated arginase activity when manganese was present.) When 0.6 mM citrulline was added to the complete medium, containing arginine, arginase activity was not affected, excluding the possibility that citrulline acted directly as an inducer of arginase.

FIG. 6. A, effect of proline on development of arginase during a 4-day growth cycle in normal medium containing 0.1 mM Mn++ (●●●●●). Proline, at 6 mM concentration, was added at the time shown by the arrow. The control experiment without proline is indicated by ○○○○○. B, effect of proline on development of arginase during a 4-day growth cycle in complete medium containing also 6 mM leucine and 0.1 mM Mn++ (●●●●●). Proline at 6 mM concentration was added at the time shown by the arrow. The control experiment without proline is indicated by ○○○○○.
Effect of Ornithine—In the absence of manganese, ornithine added at 6 mM concentration to the growth medium had no significant effect on the arginase activity of the Chang’s cells. In the presence of manganese, ornithine had a stimulating effect; this was not observed when proline was added also (Fig. 9, A and B). Ornithine is a competent stabilizer of arginase in vitro. However, the lack of effect by ornithine in the absence of manganese suggests that this amino acid stimulates enzyme synthesis and does not act by stabilizing the enzyme. Since ornithine δ-transaminase is strongly inhibited by excess substrate in vitro (40), the effect of ornithine at a high concentration may be of the same nature as proposed earlier for the effects of leucine and valine.

Glycine—Glycine, another competent stabilizer of arginase in vitro (44), added at a 6 mM concentration to the medium, had no stimulating effect on the arginase activity of the cells.

DISCUSSION

The present study has shown that an increased concentration of lysine, leucine, valine, or ornithine in the growth medium increased arginase activity in Chang’s liver cells. These amino acids are known to inhibit either arginase or ornithine δ transaminase, the enzymes which catalyze the first two steps in the conversion of arginine to proline. An increase in arginase was also brought about by o-aminobenzaldehyde, which can react with Δ₁-pyrroline 5-carboxylate, a metabolite of the same reaction chain. Finally, a substitution of citrulline for arginine in the

FIG. 7. A, effect of proline on the specific activity of arginase in Chang’s liver cells grown in normal complete medium which was serially diluted every 2nd day at the times indicated by the arrows. Proline was either maintained at 6 mM concentration throughout (●—●) or not added at all (O—O). B, cell concentration in the experimental flasks corresponding to the experiment described in A.

FIG. 8. Specific activity of arginase in Chang’s liver cells during a 3-day growth period in an otherwise normal medium lacking arginine and containing in addition 0.6 mM citrulline and 0.1 mM Mn⁺⁺ (●—●). The control experiment with the normal complete medium with arginine and 0.1 mM Mn⁺⁺ is indicated by O—O.

FIG. 9. A, effect of ornithine (6 mM) on the specific activity of arginase in Chang’s liver cells during a 3-day growth period in a normal complete medium containing 0.1 mM Mn⁺⁺ (●—●). The control experiment without ornithine is indicated by (O—O). B, effect of addition of proline (6 mM) on the rise of activity caused by ornithine. The curve indicated by ●—● plots the specific activity of arginase in cells grown in complete medium containing in addition 6 mM ornithine. At the time indicated by the arrow, proline was added at 6 mM concentration (O—O).
growth medium, which might result in limiting the level of substrate in the reaction chain, also brought about a stimulation of arginase activity. On the other hand, a decrease in arginase activity was shown following addition to the medium of proline, a product of the same reaction sequence.

Experiments in which protein synthesis was inhibited by puromycin indicated that the rate of accumulation of enzyme rather than activation or inactivation of preformed enzyme molecules was involved. These results strongly suggest that the arginase level is regulated by a negative feedback control, in which a product of the reaction chain, initiated by the arginase-catalyzed reaction, depresses the synthesis of the enzyme.

During protein turnover, which is a continuous process in animal cells, the level of individual enzymes must depend on the relative rates of both synthesis and degradation, and specific regulation of an enzyme could be brought about by modifications either of the rate of synthesis or of degradation of the enzyme (45). The fact that leucine, valine, ornithine, and proline affected the arginase level of Chang's liver cells without affecting cell growth suggests that they can influence arginase accumulation specifically by a mechanism other than regulation of total protein synthesis. The increase in arginase activity brought about by lysine, leucine, valine, or omega-aminobenzaldehyde in the presence as well as in the absence of manganese ions indicates that this increase of enzymatic activity is of a different nature than the stabilization caused by manganese. This conclusion is further supported by the observation that glycine, which stabilizes the enzyme in vitro (but has little effect on the reaction sequence from arginine to proline), did not affect arginase activity. It also seems unlikely that the repressing effect of proline is the result of a labilization of the enzyme.

Although definite mechanisms of regulation of arginase activity in cells in tissue culture cannot yet be proposed, the present experiments strongly suggest that changes in the concentration of specific individual amino acids in the medium modify the rate of synthesis of the enzyme, and that arginase synthesis is regulated by product repression in a manner analogous to the regulation of many bacterial enzymes (46).

Regulation of arginase synthesis by product repression is, of course, not inconsistent with other results showing an induction of this enzyme by its substrate (24-29). It is evident, however, that, if arginase is repressed by a product of the enzyme reaction, addition ofarginine might also affect accumulation of the repressor. Earlier results on arginase induction in tissue cultures have shown variations of response to arginine in different cell strains (25, 27) and in the same cell strain under different growth conditions (29).

One might speculate whether proline is actually a corepressor. This amino acid could increase the intracellular accumulation of Δ1-pyrroline 5-carboxylate since proline inhibits both Δ1-pyrroline 5-carboxylate reductase (47) and Δ1-pyrroline 5-carboxylate dehydrogenase (22). Δ1-Pyrroline 5-carboxylate seems to be in a strategic position for a regulator metabolite at the branch point of the reaction chain. The lack of effect of externally added Δ1-pyrroline 5-carboxylate might be due to the lability of the compound (41).

A comparison between the variations of arginase activity in Chang's cells with variations in the activity of ornithine Δ-transaminase reported in the previous paper (23) indicates that the two enzymes are not coordinately regulated. Although leucine, valine, and ornithine had similar effects on the activities of the two enzymes, addition to the growth media of lysine, omega-aminobenzaldehyde, or proline had strong effects on arginase but not on ornithine Δ-transaminase. Lack of coordination between the two enzymes may be further indicated by the observation that the level of ornithine Δ-transaminase activity in Chang's liver cells is comparable to its level in mammalian liver in vivo (23), but arginase activity of the Chang's liver cells is only 0.05 to 3% of its specific activity (in the range of 100) reported for human liver (48).

That the feedback regulation of arginase may be related to the formation of a product of ornithine via the ornithine Δ-transaminase reaction permits some interesting considerations of the coordinate regulation of urea cycle enzymes in urotelial vertebrates. The simultaneous increase in all the enzymes of the urea cycle during liver differentiation (49) seems to be initiated by an increase in carbamylphosphate synthetase. It is conceivable that the increase in ornithine transcarbamylase activity results in more effective competition with ornithine Δ-transaminase for their common substrate, resulting ultimately in a decrease in the level of repressor for arginase synthesis. Since the enzymes involved in the formation of arginine from citrulline have been shown to be repressed by arginine (27), it seems unlikely that an increase in arginase could in turn remove the repressor for these arginine-forming enzymes. The regulation of the enzymes of the complete cycle would then depend on regulation of a single critical point of the cycle, the ornithine transcarbamylase reaction.

Acknowledgments—Thanks are due Dr. Tryggve Gustafson for his valuable advice and encouragement and for providing the facilities to conduct this work. The expert technical assistance of Miss K. Arvidsson and Miss U. Hammar is gratefully acknowledged.

REFERENCES


Arginase Activity during the Growth Cycle of Chang's Liver Cells
    Eva E. Eliasson and Harold J. Strecker


Access the most updated version of this article at http://www.jbc.org/content/241/24/5757

Alerts:
    • When this article is cited
    • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/241/24/5757.full.html#ref-list-1