ORNITHINE DECARBOXYLASE AND RIBOSOMAL RNA SYNTHESIS DURING THE STIMULATION OF LYMPHOCYTES BY PHYTOHAEMAGGLUTININ

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1. Introduction

Ornithine decarboxylase (ODC) catalyses the first step in the biosynthesis of polyamines, the conversion of ornithine to putrescine [1]. Its activity rises dramatically at an early stage in the conversion of many types of cells from a non-growing to a growing state [2–4] and it has also been reported to have an unusually short half life [5]. The consequent rapid fluctuations in enzyme activity in response to growth stimulation suggest that this enzyme might be involved in cell growth regulation mechanisms.

Prominent among the sequence of metabolic changes invariably associated with the change from the non-growing to the growing state is an early increase in the synthesis of ribosomal RNA (rRNA), and there are some suggestions that ODC activity may be associated with this [6, 7]. The effect of growth stimulation on rRNA synthesis has been studied in most detail during the activation of cultured human lymphocytes by phytohaemagglutinin (PHA). Separate effects of the growth stimulant on the synthesis of ribosomal precursor RNA and on the rate and efficiency of the maturation of the precursor rRNA have been identified [8–10]. We have found that ODC activity increases greatly when lymphocytes are cultured with PHA. This increase correlates with, and is dependent upon, the major increase in the net synthesis of rRNA.

2. Materials and methods

Lymphocytes were purified from human blood and cultured at 2×10^6/ml in Eagle's medium containing 10% autologous plasma as previously described [11]. PHA batches Q1 and PM1, obtained from the Wellcome Research Laboratories, Beckenham, Kent, were both used at a concentration of 2 μg/ml, the concentration found to cause maximal incorporation of ^{3}H-thymidine into DNA 2 days later. Incorporation of ^{3}H-uridine into RNA after a 1 hr pulse and ^{14}C-phenylalanine into protein after a 2 hr pulse were determined as previously described [12].

ODC activity was determined in 10,000 *g* supernatant fractions of lymphocytes disrupted by freezing and thawing. The assay was carried out as described by Pegg and Williams-Ashman [13] except that mercaptoethanol was replaced by 5 mM dithiothreitol, and incubation was for 1 hr. Release of $^{14}CO_2$ from 1-^{14}C-L-ornithine was linear for at least 2 hr, and was proportional to the enzyme concentration.

Lymphocyte nuclei were prepared and their RNA polymerase determined essentially as described by Handmaker and Graef [14]. Incorporation of ^{3}H-UTP into RNA was determined in the presence of 0.2 M $(NH_4)_2SO_4$ and with or without 1 μg/ml α-amanitin. Amanitin at this concentration totally inhibits DNA-like RNA synthesis by RNA polymerase II without affecting rRNA synthesis by RNA polymerase I [15, 16]. α-Amanitin was kindly made available to us by Professor Th. Wieland. RNA polymerase activity was determined after incubation for 1 hr, at which time incorporation had reached a plateau, and was proportional to the number of nuclei added.

3. Results

ODC activity in resting lymphocytes was very low, and was not detectable in many experiments. Fig. 1 shows that activity stayed low for the first 6 hr after the addition of PHA, rose rapidly between 6 and 14 hr, and remained high for up to 48 hr. The lack of activity in extracts of unstimulated cells was not due to the presence of an inhibitor, as the extracts did not inhibit acitivity of extracts of PHA treated cells (table 1). Fig. 1 also shows the changes in several other parameters after the addition of PHA. There was little correlation between the rise in ODC activity and the rate of protein synthesis or the amanitin-sensitive RNA polymerase activity. The enzyme reached its maximum activity well before the initiation of DNA synthesis, which occurs at about 30 hr. The rise in ODC activity did coincide with a large increase in the rate of ^{3}H-uridine incorporation into RNA, although this increase began before and continued after the rise in ODC activity. The amanitin-resistant RNA polymerase activity also began to increase before the ODC, but most of the increase in the activities of these two enzymes occurred over the same period. Both ^{3}H-uridine incorporation [17] and amanitin-resistant RNA polymerase activity [18] are primarily a function of the rate of rRNA synthesis in this system.

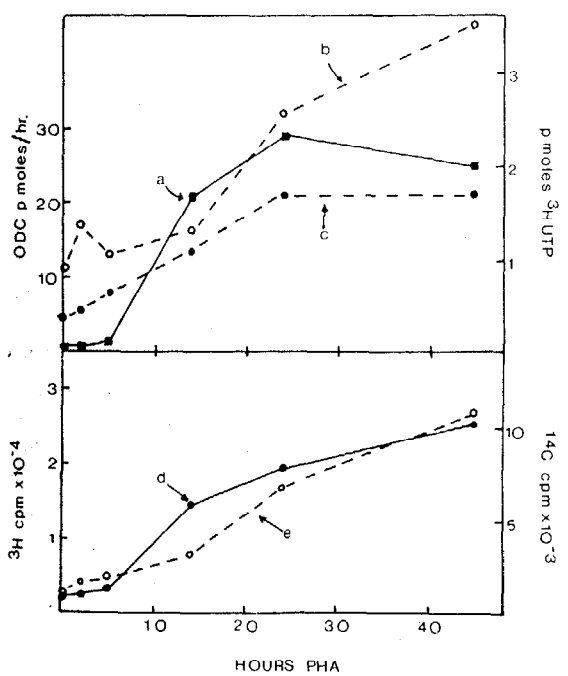

Fig. 1. Changes after the addition of PHA in (a) ODC activity, expressed as pmoles ornithine decarboxylated/10^{6} lymphocytes in 1 hr. (b) Amanitin-sensitive RNA polymerase activity, pmoles ^{3}H-UTP incorporated into RNA/10^{6} nuclei. (c) Amanitin-resistant RNA polymerase activity, units as (b). (d) ^{3}H-Uridine incorporation into RNA, cpm/10^{6} lymphocytes in 1 hr. (e) ^{14}C-Phenylalanine incorporation into protein, cpm/10^{6} lymphocytes in 2 hr.

Table 1
Effect of co-incubation on ODC activity of unstimulated and 24 hr PHA lymphocytes.

Unstimulated lymphocytes	24 hr PHA lymphocytes	ODC[a]
5×10^{6}	–	10.8
–	5×10^{6}	74.9
5×10^{6}	5×10^{6}	78.9

Each flask contained extract derived from the number of lymphocytes indicated in a total volume of 0.5 ml.

[a] pmoles ornithine decarboxylated/flask in 1 hr.

Low actinomycin concentrations can inhibit selectively the synthesis of rRNA [19, 20]. Selective inhibition of rRNA synthesis throughout the first 24 hr of the response of lymphocytes to PHA allows many of the usual morphological changes to occur, and also a marked increase in the rate of protein synthesis [20]. The increase in ODC activity was as exquisitely sensitive to actinomycin as the synthesis of rRNA, as judged by the amanitin-resistant RNA polymerase activity (table 2) and also by the rate of ^{3}H-uridine incorporation. The rate of incorporation of ^{14}C-phenylalanine into protein and the activity of the amanitin-sensitive RNA polymerase were much less affected. The concentrations of actinomycin do not prevent the induction or uridine kinase in this system, which otherwise shows kinetics similar to ODC [17].

ODC appears to be a most unstable enzyme. Its activity declined with a half life of about 15 min when cycloheximide was added to stimulated lymphocytes, a value comparable to that obtained in other systems [5]. However, when low or high concentrations of actinomycin were added to lymphocyte cultures that had been preincubated with PHA for 24 hr, ODC activity was not greatly affected for 1–2 hr, while rRNA synthesis decreased dramatically (table 3). Syn-

Table 2
Effect of low actinomycin concentrations on ODC and amanitin-resistant RNA polymerase activities, and on the rate of incorporation of ^{14}C-phenylalanine into protein.

PHA	Actinomycin (μg/ml)	ODC[a]	Amanitin-resistant RNA polymerase[b]	^{14}C-Phenylalanine
–	–	0.9	0.25	468
+	–	14.1	0.95	2950
+	0.01	2.3	0.35	2283
+	0.025	1.6	0.20	2117

Actinomycin and PHA were added at the same time, and cultures were harvested 24 hr later. Incorporation of ^{14}C-phenylalanine into protein was determined during a 2 hr pulse from 22 to 24 hr after the addition of PHA.

[a] pmoles ornithine decarboxylated/10^6 lymphocytes in 1 hr.
[b] pmoles ^{3}H-UTP incorporated into RNA/10^6 nuclei.
[c] cpm ^{14}C-phenylalanine incorporated into protein/10^6 lymphocytes.

Table 3
Effect of high or low actinomycin concentrations added 24 hr after PHA and 2 hr before termination of culture on ODC and amanitin-resistant RNA polymerase activities and on ^{3}H-uridine incorporation into RNA.

Actinomycin (μg/ml)	ODC[a]	Amanitin-resistant RNA polymerase[b]	^{3}H-Uridine[c]
None	17.1	1.14	64324
5	14.0	0.14	692
0.05	13.2	0.24	34093

[a,b] Units as in table 2.
[c] cpm ^{3}H-uridine incorporated into RNA during a 1 hr pulse from 25.5 to 26.5 hr after the addition of PHA.

thesis of the enzyme, once initiated, thus seems not to require concomitant rRNA synthesis, at least in the short term.

4. Discussion

These results show that stimulation of lymphocytes leads to a marked increase in ODC activity. The similarity of these results to those obtained in other systems in which resting cells are stimulated to enter cell division emphasises the fundamental similarity in the biochemical sequence of responses to growth stimulation in these different systems. The rise in this activity was not one of the earliest changes in lymphocyte metabolism in response to PHA, but occurred in the mid-prereplicative phase and was maintained through the initiation of DNA synthesis.

Both the time of appearance of this activity and, more especially, its sensitivity to low concentrations of actinomycin, suggest that it may be associated with the increase in the synthesis of rRNA. Table 3 shows that the selective inhibition of rRNA synthesis by actinomycin is not secondary to effects on ODC. Taken with table 2, this implies that the rise in ODC activity is a function of the rise in the rate of synthesis of rRNA rather than vice-versa.

Within 6 hr of the addition of PHA to lymphocytes there is some increase in the rate of rRNA precursor synthesis, a considerable reduction in the time required for the maturation of rRNA precursors and an increase in the efficiency of precursor maturation from below 50% to almost 100% [8–10]. ODC activity remains very low throughout this period. However, the major increase in the rate of rRNA synthesis, as judged by ^{3}H-uridine incorporation and the amanitin-resistant RNA polymerase activity, occurs between 6 and 24 hr after PHA is added, after these rapid initial changes. The increase in ODC activity parallels this and may thus be related to the net rRNA synthesis which results from these large increases in the rates of synthesis and processing of rRNA.

It should be remembered that the activity determined here is that of the isolated enzyme, but that in the cell its activity will most likely also be affected by the concentration of substrate and product, and possibly other inhibitors and activators. The situation

may thus be analogous to the case of uridine kinase, in which activity is first increased by activating existing enzyme, and only later by an increase in the amount of enzyme present [17].

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