ORNITHINE DECARBOXYLASE ACTIVITY IN UNINFECTED AND VACCINIA VIRUS-INFECTED HELA CELLS

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SUMMARY: Ornithine decarboxylase activity increased markedly in both uninfected and vaccinia virus-infected HeLa cells following their transference to maintenance medium. The maximum level of activity in virus-infected cells was determined by the multiplicity of infection. These increases were completely inhibited by cycloheximide and partially inhibited by actinomycin D. In virus-infected cells only, treatment with FUdR resulted in prolonged expression of enzyme activity. The Km values for ornithine decarboxylase in uninfected and vaccinia virus-infected HeLa cells were significantly different. The mechanisms that may effect these changes are discussed.

Many studies have shown a marked stimulation of ornithine decarboxylase (E.C.4.1.1.17) in growing animal tissues (1). This enzyme catalyzes the formation of putrescine, the initial step in polyamine biosynthesis, and significant increases in the polyamine content of growing animal tissues have been described (2). The basic polyamines have been shown to interact with acid macromolecules and to affect DNA replication, RNA synthesis and the synthesis of proteins by ribosomes (3). These properties suggest a regulatory function of polyamines on macromolecular synthesis (4). The expression of this function may be determined by changes in ornithine decarboxylase activity.

During vaccinia virus replication the infective genome modifies the metabolism of the host cell to the production of virus-specified DNA, RNA and proteins (5). This paper describes some properties of ornithine decarboxylase in uninfected and vaccinia virus-infected HeLa cells. These studies show that virus infection results in both quantitative and qualitative changes in the activity of this enzyme.

METHODS:

The laboratory line of HeLa cells used (6) was grown in Eagle's minimum essential medium (MEM) containing 5% calf serum. Confluent monolayers (8×10^6 cells) were either "sham" infected or infected with the Lister strain of vaccinia virus using 5 plaque-forming units (p.f.u./ml). Infectivity titres were determined by plaque assay in HeLa cell monolayers. After adsorption for

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one hour, inocula were removed and replaced by MEM containing 2% calf serum. All procedures were carried out at 37°. At various times after infection the cells from replicate samples in each series were resuspended using ice-cold 0.02% ethylenediaminetetra-acetic acid.

Ornithine decarboxylase activity in the recovered cells was determined essentially by the method of Hogan (7). DL-1-[^{14}C]ornithine monohydrochloride (specific activity 29 mCi/m-mol) was obtained from the Radiochemical Centre, Amersham, Buckinghamshire and the quantitative recovery of $^{14}CO_2$ was measured by methods described previously (8). Protein estimations were made using a biuret-phenol method (9).

Actinomycin D was used at a concentration of 1.0 µg/ml, cycloheximide at 20 µg/ml and 5-fluorodeoxyuridine (FUdR) at 2.5 µg/ml. These concentrations of inhibitors prevented the replication of vaccinia virus in the cell system used but without overt cytotoxic effect on uninfected cells.

RESULTS:

Ornithine decarboxylase activity increased markedly in both uninfected and vaccinia virus-infected HeLa cells following their transference to maintenance medium (Fig. 1A). Enzyme activity was not above the background of the assay system for the first hour but after this time activity increased rapidly. In uninfected cells maximum activity was attained at 5 hrs and this level was maintained until 6 to 7 hrs before declining rapidly. Although a similar

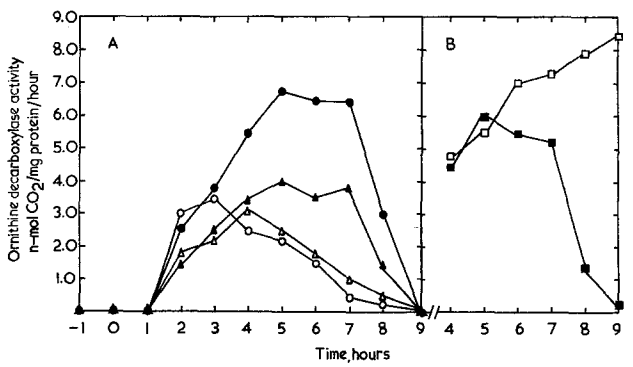

Figure 1. Ornithine decarboxylase activity from uninfected and vaccinia virus-infected HeLa cells. At time = -1 hr, confluent HeLa monolayers were either "sham" infected or infected with vaccinia virus using 5 p.f.u./cell. At time = 0 hr, inocula were removed and the cell cultures were transferred to appropriate maintenance media. A : enzyme activity in uninfected cells, ▲—▲; enzyme activity in infected cells, ●—●; enzyme activity in uninfected cells in the presence of 1.0 µg/ml actinomycin D, Δ—Δ; enzyme activity in infected cells in the presence of 1.0 µg/ml actinomycin D, o—o. B : enzyme activity in infected cells, ■—■; enzyme activity in infected cells in the presence of 2.5 µg/ml FUdR, □—□.

Table 1. Effect of multiplicity of infection on ornithine decarboxylase activity in vaccinia virus-infected HeLa cells

Multiplicity of infection (p.f.u./cell)	Enzyme activity* (at 6 hrs post-infection)
0	4.66
0.5	4.51
2.5	5.00
5.0	7.33
12.5	3.55
25.0	2.68
50.0	2.12

*n-mol CO_2/mg protein/hr

temporal pattern was observed in virus-infected cells the level of enzyme activity at 5 hrs was significantly higher than that in uninfected cells.

Further experiments showed that ornithine decarboxylase activity in virus-infected cells was affected quantitatively by the multiplicity of infection (Table 1). At 5 p.f.u./cell the enzyme activity was 40% greater than uninfected cells. Higher inocula resulted in a progressive reduction in enzyme activity to levels less than uninfected cells. In all experiments the enzyme activity in virus-infected cells declined rapidly within one or two hours of reaching maximum levels.

Qualitative differences in the mechanisms determining the increased enzyme activities observed were indicated by the effect of inhibitors of translation, transcription and DNA replication. Cycloheximide inhibited completely the increase in enzyme activity in both uninfected and infected cells. In the presence of actinomycin D there was an initial increase in enzyme activity that continued until 4 hrs in uninfected cells but until 3 hrs only in virus-infected cells. After these times the enzyme activities declined rapidly (Fig. 1A). The earlier decay of ornithine decarboxylase activity in virus-infected cells in the presence of actinomycin D was observed consistently. Treatment with FUdR did not inhibit the increase in enzyme activity and the temporal pattern in uninfected cells was unaltered. However, the level of activity in virus-infected cells continued to increase from 5 hrs unlike cells infected in the absence of the inhibitor (Fig. 1B).

The reaction velocities of ornithine decarboxylase in uninfected and virus-infected cells were determined in the presence of increasing ornithine concentrations. The Km values were calculated by the method of Lineweaver & Burk (10) : typical results are shown in Figure 2. From eight separate determinations the mean Km value, with standard deviation, was $0.12 \pm 0.03 \times 10^{-3}$ M for the enzyme

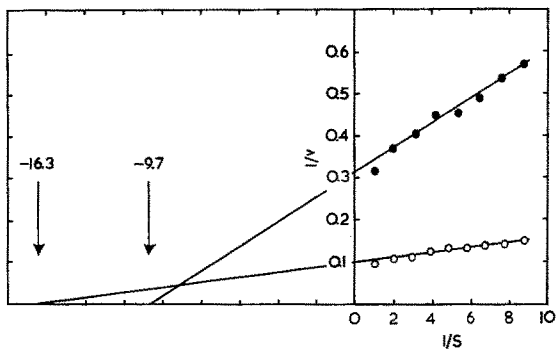

Figure 2. Reaction rates of ornithine decarboxylase activity from uninfected and vaccinia virus-infected HeLa cells determined at different ornithine concentrations. The data are plotted according to the method of Lineweaver and Burk (10) v is n-mol of CO_2 released/hr and S is the concentration of ornithine (mM). Enzyme activity in uninfected cells, ●——●; enzyme activity in infected cells, o——o.

from uninfected cells but $0.05 \pm 0.01 \times 10^{-3}$ M from infected cells. These results show significant differences in ornithine decarboxylase in uninfected and vaccinia virus-infected HeLa cells.

DISCUSSION:

The increased ornithine decarboxylase activity in uninfected HeLa cells differs in some respects from similar studies made in other cell systems. In human lymphocytes this enzyme activity can be increased by the addition of non-essential amino acids to the culture medium (11). The addition of serum to cultures of BHK 21 cells in serum-depleted medium has been shown to stimulate ornithine decarboxylase activity (12). In the present study, the culture medium used contained essential amino acids only and similar increases in enzyme activity were observed in the absence of serum (unpublished results). It is significant, perhaps, that medium replenishment stimulates macromolecular synthesis in confluent monolayers of the HeLa cells used (6). Inhibition of DNA synthesis did not affect the increase nor the temporal pattern of enzyme activity. However, this increase is dependent upon both RNA and protein synthesis as reflected by the partial inhibition by actinomycin D and complete inhibition by cycloheximide. These inhibitors have been shown to have similar effects on the same enzyme activity in rat hepatoma (HTC) cells (7).

Several features suggest that different mechanisms affect the expression of ornithine decarboxylase activity following infection with vaccinia virus. Host-directed protein synthesis declines rapidly in vaccinia virus-infected cells reaching minimum levels by 5 hrs post-infection (5). Studies on ornithine

decarboxylase in other cell systems have shown that this enzyme has a very short half-life (1). In vaccinia virus-infected HeLa cells, therefore, expression of the host-specified enzyme should be inhibited. However, enzyme activity was enhanced in virus-infected cells and affected quantitatively by the multiplicity of infection. This increased activity required protein synthesis but an additional requirement for RNA synthesis is shown by the reduced enzyme activity in the presence of actinomycin D. This inhibitor effected an earlier decay of enzyme activity in virus-infected cells indicating either quantitative or qualitative changes in transcription. A qualitative change is indicated by the different effect of FUdR since inhibition of DNA synthesis in virus-infected cells resulted in a prolonged expression of enzyme activity. These results show ornithine decarboxylase activity in vaccinia virus-infected cells depends upon both protein and RNA synthesis but is controlled to some extent by DNA synthesis.

Previous studies have shown that vaccinia virus infection induces other enzyme activities (5, 6). Their expression requires both translation and transcription regulated by virus-specific DNA synthesis. The physico-chemical characteristics of these enzymes differ from similar activities in the host cell suggesting that the induced enzymes are coded by the virus genome. Identical requirements are shown for the expression of ornithine decarboxylase in virus-infected cells. Further, comparison of the Km values shows significant differences between the enzyme activity in uninfected and virus-infected cells. It is concluded that virus-specified mechanisms determine increased ornithine decarboxylase activity in vaccinia virus-infected HeLa cells.

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