DOES CYCLOHEXIMIDE INTERFERE WITH PROTEIN DEGRADATION?

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SUMMARY

The presence of the protein synthesis inhibitor cycloheximide does not affect the rate of general protein degradation (turnover), in growing as well as in resting cultured KB cells.

INTRODUCTION

Cycloheximide, a well-known inhibitor of protein synthesis (1, 2) is frequently employed in order to determine the rate of degradation of proteins. We recently applied this drug to two established cell lines (3) and found that several enzymes are relatively stable in the absence of their synthesis. Kenney (4) recently reported experiments on the rate of turnover of tyrosine transaminase in rat liver which suggested that cycloheximide does inhibit not only the synthesis of proteins but also their degradation. It has also been observed that the half life of this enzyme in a cultured cell line amounts to 7—8 hrs when determined by cycloheximide, while a half life of 1—2 hrs is observed when the inducer is removed (5).

The question stands to what extent is this inhibitory effect on protein

degradation general. In the experiments described below we test whether the presence of cycloheximide inhibits the degradation of all cell proteins, i.e., affects protein turnover in cultured cells. It is found that the rate of disappearance of label from labeled cell protein is unaffected by the presence of cycloheximide, in growing as well as in nongrowing cells. It seems that the effect of cycloheximide on protein degradation is limited to a special class of systems and proteins.

PROCEDURE

The KB cells were the same as in previous experiments (3). They were grown in 60-mm plastic dishes under 10% CO_2 at 37°, in Eagle's medium with 5% horse serum. Cells with labeled proteins were obtained by seeding 10^5 cells in medium containing 1 μc/ml ^{14}C-L-leucine (Amersham preparation CFA.273, final specific activity in the medium 1.25 μc/mmole). A fresh portion of the same medium was given after 3 days, and the experiment was started on the 6th day. By that time there were about 2.25 x 10^6 cells/plate, so that over 95% of the cell protein must have been labeled, ensuring uniform labeling of all proteins. On the 6th day the radioactive medium was removed, the plates were washed, and unlabeled medium with the desired concentration of cycloheximide (Calbiochem) was given. The loss of label from protein was determined at fixed intervals as follows: The medium was collected and the plate was washed twice with phosphate buffered saline (PBS) containing 1% leucine. The cells were collected with a policeman into 1 ml PBS and sonicated for 10". Protein in 0.1 ml was determined by the Lowry method. 0.5 ml were added to 0.5 ml 10% TCA and held for 10' in boiling water. The coagulated precipitate was centrifuged, washed twice in 1% TCA—1% leucine, dissolved in 0.5 ml NaOH 0.1 M and counted in Bray's counting solution.

The samples were counted in a Packard Model 3320 liquid scintillation counter, and the number of decompositions/min was determined by external standardization.

RESULTS

The rate of protein degradation, in terms of total activity (dec/min lost per plate) as well as specific activity (dec/min lost per mg protein) is shown in Figs. 1a and 1b. Both plots are required because of the following complication: In control cultures, receiving no cycloheximide, specific activity decreases not only because of turnover but also because of new protein formed during cell replication. The decline in total activity gives here the best measure of the rate of protein degradation. In contrast, cells which receive the inhibitor do not only cease to synthesize proteins but also lose counts gradually, because of cell deterioration and death (in addition to the loss by turnover). Cell death contributes to the change in total activity but not to the change in specific activity; therefore the specific activity is the proper measure of cell protein degradation by turnover in these cells. This can be readily shown in formal terms, assuming that degradation associated with turnover is an exponential function of time.

In the experiment shown in Fig. 1, the rate of protein degradation at two concentrations of cycloheximide is compared with control cultures which received no inhibitor. The higher dose, 10 μg/ml, inhibits 95% of protein synthesis (3) but leads to considerable cell deterioration after 1-2 days. The low dose inhibits protein synthesis by only 35% but cells stay intact throughout most of the experiment, without further replication. This is reflected in Fig. 1; the total activity (lower curve) of cultures with 2 μg/ml decreases at the same rate as control cells (no cell death) but the specific activity (upper curve) decreases as in cells with 10 μg/ml (no dilution of protein by growth).

Thus, the slope of the upper curve gives the turnover rate in the control cells,

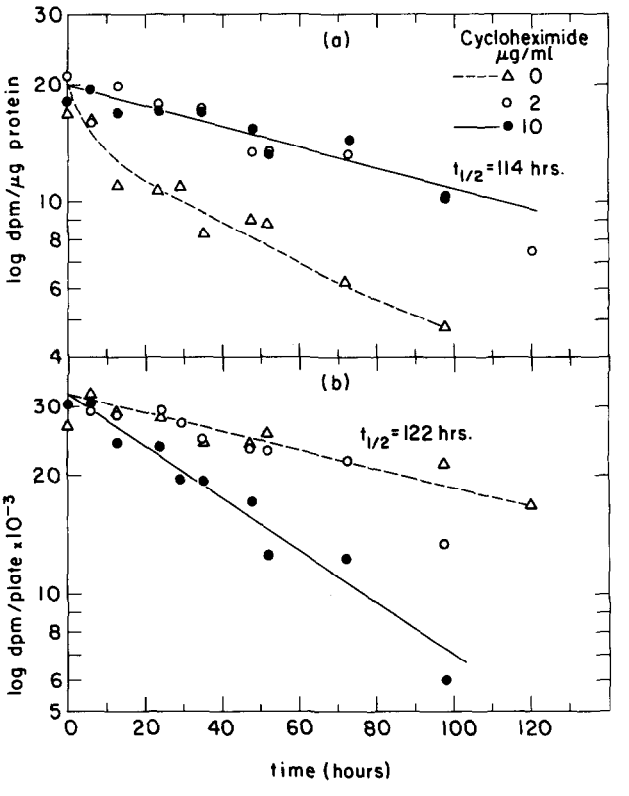

Fig. 1. The disappearance of label from ^{14}C-L-leucine labeled replicating KB cells. The cells were grown for 6 days in medium containing labeled leucine. At t = 0, nonradioactive medium containing cycloheximide as stated in the figure was given. At indicated times, the radioactivity of cell protein was determined, as described in the text.

Upper curve: The decrease in total activity. Lower curve: The decrease in specific activity.

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while the slope of the lower curve gives the turnover rate in the cells with cycloheximide. The values for the half time of protein turnover are found to be 121 hrs in control cells, a figure which is in good agreement with the value reported originally for KB cells by Eagle and co-workers (6). The half life of protein degradation in the presence of cycloheximide, as judged from the decrease in the presence of cycloheximide, is $t_{1/2}$ = 114 hrs. This value is very close to the value of $t_{1/2}$ of normal turnover, and leads to the conclusion that inhibition of protein synthesis by cycloheximide does not interfere with the normal rate of protein degradation.

To further substantiate this conclusion, it is desirable to measure turnover under conditions where the complication caused by growth of control cells is absent. Growth can be inhibited in a number of ways. Omission of a nutrient like an essential amino acid is undesirable because it may induce the formation or destruction of special proteins. The inhibitory effect of radiation and the lack of serum are not well understood. The inhibition of replication by hydroxy-urea, an inhibitor of deoxynucleotide biosynthesis (7) was found to be satisfactory, and a dose of 40 μg/ml was found to inhibit replication completely (as judged by cell counts) with little toxic effect. The rate of protein degradation in labeled cells to which hydroxy-urea was given together with cycloheximide is shown in Fig. 2. The half times for the disappearance of specific activity were 51, 44 and 38 hrs when 10, 2 and 0 μg/ml cycloheximide were present, respectively. The reason for the higher rate of turnover in this experiment is not clear; it may be connected with the presence of hydroxy-

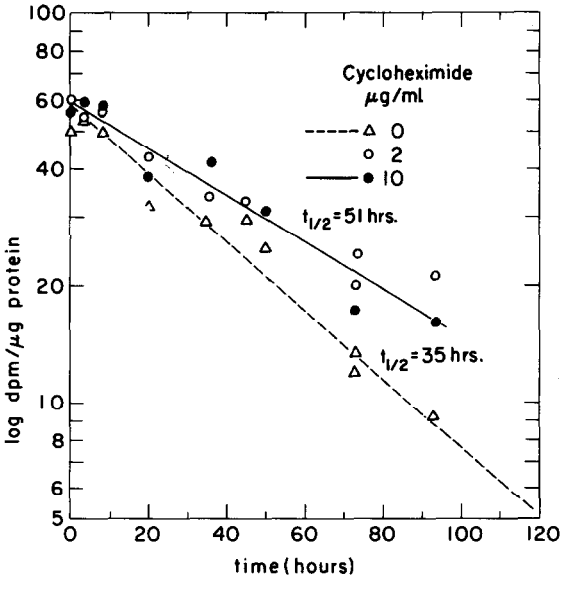

Fig. 2. The disappearance of label from ^{14}C-L-leucine labeled KB cells in the absence of replication. The same procedure as in Fig. 1 was employed, only that the nonradioactive medium contained also 40 μg/ml hydroxy-urea.

urea. The somewhat lower half time observed in the absence of cycloheximide is most probably due to the slight increase in protein content (about 60% in 3 days) occurring in the absence of replication. In any case, it is clear that cycloheximide causes no general inhibition of protein degradation even under conditions of limited growth.

In conclusion, the inhibitory effect of cycloheximide on some regulatory enzymes (8, 9) cannot be general, and probably operates through mechanisms specific to each system. Organs other than liver may be the target of the protein synthesis inhibitor, and evidence that the hypophysis is involved is available (8).

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