THE EFFECT OF TOSYL LYSINE CHLOROMETHYL KETONE ON THE ACTIVITY OF URIDINE DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE OF THE CELLULAR SLIME MOLD DICTYOSTELIUM DISCOIDEUM

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SUMMARY. We report here that the specific activity of UDPG pyrophosphorylase in extracts of D. discoideum amoebae and preculmination-stage cells increases as a function of the length of their exposure to tosyl lysine chloromethyl ketone, an irreversible inhibitor of a number of serine and sulfhydryl proteases. This compound also stabilizes the activity of the enzyme in crude extracts of amoebae. These results can be interpreted, with some assumptions, as evidence in support of the hypothesis that the levels of the enzyme are maintained in D. discoideum by a balance of synthesis and degradation.

INTRODUCTION. The specific activity of D. discoideum UDPG pyrophosphorylase (EC 2.7.7.9) remains constant during vegetative growth and the aggregation stage, but then increases dramatically between the aggregation and culmination stages. The observed increase in the specific activity of the enzyme is the result of a parallel increase in enzyme protein (1-4). Sussman and co-workers have asserted that UDPG pyrophosphorylase accumulation is due to induction of transcription and translation (5-7). Wright and co-workers, however, dispute this claim. They believe that both before and during differentiation, the level of the enzyme is maintained by a balance of synthesis and degradation. They have proposed that the accumulation of the enzyme is due to a decrease in the rate of its degradation (3, 4, 8).

It has been demonstrated that tosyl lysine chloromethyl ketone is an effective irreversible inhibitor of a number of serine and sulfhydryl proteases (9-12). The purpose of this study was to determine the effects of TLCK[1] on UDPG pyrophosphorylase in a crude cell extract and on the specific activity of the enzyme during development. It was hoped that the results of

[1] Abbreviations used: TLCK, tosyl lysine chloromethyl ketone; LPS, Lower Pad Solution.

this study would support one or the other of the two mechanisms that have been proposed for the regulation of UDPG pyrophosphorylase levels in D. discoideum.

EXPERIMENTAL. Incubation of amoebae with TLCK. One ml aliquots of $2x10^8$ cells (NC-4) were plated out on 100 mm 2% agar plates. The experimental plates contained 1 mM TLCK. Control and experimental plates were incubated at 22°C. Cells were harvested with cold water at zero time and at 30 min. intervals thereafter for 180 min. The harvested cells were frozen for later assay.

Incubation of preculmination-stage cells with TLCK. One-half ml aliquots of 10^8 amoebae were pipetted onto 42.5 mm Whatman #50 filter paper discs supported by absorbant pads that had been soaked in Sussman's Lower Pad Solution (5). When the cells had differentiated to the preculmination stage (Fig. 2, hour 14) some (experimentals) were transferred, on their filter discs, to absorbant pads that had been soaked in LPS that was 1 mM in TLCK. At this time, and at one-hour intervals thereafter (Fig. 2, hours 14-17), the cells on three experimental and three control pads were harvested and frozen for later assay. After three hours of exposure to TLCK (Fig. 2, hour 17) the remaining experimental cells were transferred, on their filter discs, back to LPS-soaked absorbant pads that lacked TLCK. At one-hour intervals thereafter for the next two hours (Fig. 2, hours 17-19), the cells on three experimental and three control pads were harvested and frozen for later assay.

Preparation and assay of cell extracts. The frozen cells were thawed in 0.1 mM UDPG, and were immediately centrifuged at 39,000xg for 15 min. at 2°C. The supernatant fractions obtained from experimental and control cells treated in this manner were immediately assayed for UDPG pyrophosphorylase activity according to the method of Franke and Sussman (1). A unit of enzyme was defined as that amount of enzyme which catalyzed the conversion of one mumole of UDPG to UTP and Glucose-1-P per minute. Protein concentrations were determined by the method of Lowry et al. (13).

The effect of TLCK on the stability of UDPG pyrophosphorylase in crude extracts of amoebae. Two approximately equal quantities of amoebae (1.5×10^9) were suspended in 2.0 ml volumes of Tricine-NaOH buffer, pH 7.6. In one

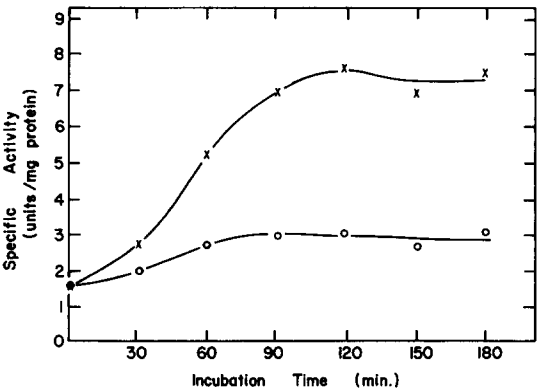

Fig. 1. The effect of 1 mM TLCK on UDPG pyrophosphorylase specific activity during the first three hours of differentiation. (-o—o-) Control cells; (-x—x-) Cells exposed to 1 mM TLCK.

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of the samples (experimental), the buffer was 1 mM in TLCK. Both suspensions were immediately frozen. UDPG pyrophosphorylase activity was extracted from each sample as described above. Each extract was then incubated at 22°C. At zero-time and at 20 min. intervals thereafter for 100 min., 0.1 ml aliquots were removed from each sample and immediately assayed for UDPG pyrophosphorylase activity.

RESULTS. As shown in Fig. 1, exposure of D. discoideum amoebae to TLCK produces a dramatic increase in the specific activity of UDPG pyrophosphorylase. The specific activity of the enzyme in extracts of amoebae exposed to 1 mM TLCK increased with increasing duration of exposure, and reached a maximum value that was approximately 2.5 fold greater than that of controls. The specific activity of the enzyme extracted from the control cells remained approximately constant throughout the incubation period, as would be expected at this stage of development.

Also as expected, the specific activity of UDPG pyrophosphorylase in extracts of control untreated cells increased between the 14th and 19th hours of differentiation (Fig. 2). However, the specific activity of the enzyme in extracts of preculmination-stage cells that were exposed to TLCK (Fig. 2, hours

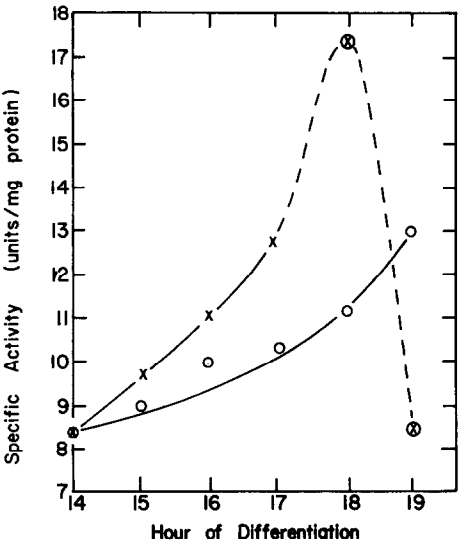

Fig. 2. The effect of 1 mM TLCK on UDPG pyrophosphorylase specific activity during preculmination. (-o— o-) Control cells; (-x—x-) Cells exposed to 1 mM TLCK between the 14th and 17th hour of differentiation; (-●--●-) TLCK-exposed cells transferred back to pads that lacked TLCK.

14-17) increased at a greater rate than that observed for control cells. Especially noteworthy are the results obtained after the TLCK-exposed cells were transferred back to pads that lacked TLCK (Fig. 2, hours 17-19). The specific activity of the enzyme in extracts from these cells continued to climb and reached a maximum value approximately one hour after the transfer. Within the second hour after the transfer, however, the specific activity of the enzyme rapidly declined to a value that was lower than that measured for the enzyme from control cells harvested at the same point in time. It should be noted that in the presence of 1 mM TLCK, cells at any stage of differentiation proceed through development normally, but at a slightly slower rate than unexposed cells.

The UDPG pyrophosphorylase activity in a buffer extract of amoebae rapidly declined upon incubation at 22°C (Fig. 3). After one hour, only about 40% of the original activity remained. In contrast, the UDPG pyrophosphorylase activity that was extracted from amoebae with buffer that was 1 mM in TLCK remained essentially constant for at least 100 min.

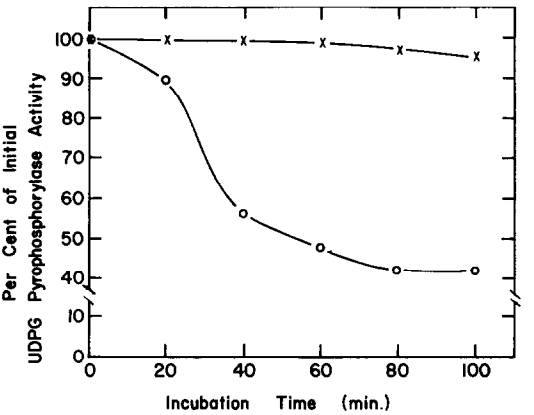

Fig. 3. The effect of 1 mM TLCK on UDPG pyrophosphorylase activity in crude extracts of amoebae incubated at 22°C. (-o—o-) Control extract; (-x—x-) Extract containing 1 mM TLCK.

DISCUSSION. The instability of UDPG pyrophosphorylase and other enzymes in crude extracts of D. discoideum, especially extracts of the early stages of differentiation, has been noted by others (1, 14, 15). Wright has suggested that enzyme instability in these cell extracts is due to the presence of one or more "destructive enzymes" (16). The TLCK-dependent in vitro stability of UDPG pyrophosphorylase observed in these experiments suggests that there is a serine or sulfhydryl protease present at least in amoebae.

The observed TLCK-induced increases in the specific activity of UDPG pyrophosphorylase and TLCK-dependent stability of the enzyme in a crude cell extract may be relevant to the mechanism by which the enzyme is accumulated in vivo. If it is assumed that there is a protease in the cells that can be inactivated by exposure of the intact cells to TLCK, and that this protease can degrade UDPG in vivo as well as in vitro, then the accumulation and subsequent decay of UDPG pyrophosphorylase activity in TLCK-exposed cells that we have observed would be consistant only with Wright's hypothesis - namely, that UDPG pyrophosphorylase is synthesized throughout growth and development, and that its accumulation during development is due to a decrease in the rate of its degradation. We are presently attempting to verify the above assumptions.

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