Stability In Vitro of Uridine Diphosphoglucone Pyrophosphorylase in *Dictyostelium discoideum*

BARBARA E. WRIGHT AND DONNA DAHLBERG

Department of Developmental Biology, Institute of Biological and Medical Sciences, Retina Foundation, Boston, Massachusetts 02114

Received for publication 4 January 1967

The stability of uridine diphosphoglucone pyrophosphorylase was examined in extracts prepared at different stages of development in *Dictyostelium discoideum*. In the early stages, the kinetics of inactivation were nonlinear, and, therefore, it was not possible to determine the specific enzyme activity. In the later stages of development, the enzyme was stable, but it could be rapidly inactivated by a heat-labile inhibitor present in extracts at an early stage.

Previous reports from this laboratory have indicated that, in extracts prepared at the early stages of development, uridine diphosphoglucone (UDPG) pyrophosphorylase is unstable (3, 6). When prepared at the later stages of development, however, the enzyme is stable. Such differential effects in vitro, as a function of the stage of morphogenesis, can be very misleading with respect to extrapolations concerning enzyme levels in vivo. Differential enzyme inactivation in the cellular slime mold has now been found for six enzymes: isocitric dehydrogenase, glucose-6-phosphate dehydrogenase (5), pyruvate kinase (Cleland and Coe, Biochim. Biophys. Acta, *in press*), cell wall glycogen synthetase (7), cellulase (Rosness, *unpublished data*), and UDPG pyrophosphorylase. This report deals with the kinetics of the inactivation of UDPG pyrophosphorylase caused by the presence of a heat-labile inhibitor at the early stages of differentiation. Other enzymes studied in the cellular slime mold are not subject to differential inactivation. These enzymes include glutamic acid dehydrogenase, alanine-α-ketoglutaric transaminase, lactic acid dehydrogenase, alanine phosphatase, and glycogen synthetase (5, 6, 9).

**MATERIALS AND METHODS**

Uridine-5’-triphosphate (UTP) and UDPG were obtained from Sigma Chemical Co., St Louis, Mo. Glucose-6-phosphate dehydrogenase (from yeast), phosphoglucomutase (from rabbit skeletal muscle), and nicotinamide adenine dinucleotide phosphate (NADP) were obtained from Calbiochem, Los Angeles, Calif.

Preparation of enzyme. *Dictyostelium discoideum* was grown on a rich medium in the presence of *Escherichia coli* and transferred at the amoeba or aggregation stage to sheets of 2% agar containing 0.01 M phosphate buffer (pH 6.5) and 0.001 M ethylene-diaminetetraacetic acid, disodium salt (EDTA) (1). At the desired stage of development, cells were harvested in 0.01 M tris(hydroxymethyl)aminomethane (Tris), pH 8.5, frozen and thawed (or passed through a French pressure cell at 30,000 psi), and centrifuged for 5 min at 12,000 × g; the supernatant fluid was assayed immediately for UDPG pyrophosphorylase activity in the direction of glucose-1-phosphate formation from UDPG (2). Into a quartz microcell of 1-cm light path was pipetted 0.575 ml of a solution of 4 × 10⁻³ M Tris buffer (pH 8.5), 1.2 × 10⁻³ M MgCl₂, and 2 × 10⁻³ M cysteine (freshly neutralized). To this was added 10 μl of 0.05 M NADP, 5 μl of glucose-6-phosphate dehydrogenase (160 enzyme units/ml), 10 μl of phosphoglucomutase (20 enzyme units/ml), 10 μl of 0.1 M UDPG, and 2 to 10 μl of extract. The reaction was started by the addition of 10 μl of 0.1 M inorganic pyrophosphatase, and the increase in absorbancy at 340 μm (A₃₄₀) was followed on a Gilford recorder (Gilford Laboratories, Inc., Oberlin, Ohio). The increase in A₃₄₀ was linear with time and enzyme concentration at 23 C. Protein was determined as previously described (9). One unit of enzyme catalyzes the reaction of 1 μmole of substrate per min. Specific activity is expressed as micromoles of reduced NADP (NADPH₂) formed per minute per milligram of protein.

**RESULTS**

The change in specific activity of UDPG pyrophosphorylase was examined as a function of developmental stage. The extracts prepared were assayed at 23 C: (i) immediately and (ii) after incubation at 35 C for 30 min. These two assay procedures are compared in Table 1. It can be seen that the apparent increase in specific activity during differentiation was 4.5 times greater in the preincubated series. This observation is consistent with a greater instability of the enzyme when prepared from earlier as compared to later stages of development.
Preculmination

Pseudoplasmodium

<table>
<thead>
<tr>
<th>Stage</th>
<th>Immediate assay</th>
<th>Assay after 30 min at 35 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoeba (4 hr)</td>
<td>0.067</td>
<td>0.013</td>
</tr>
<tr>
<td>Pseudoplasmodium</td>
<td>0.39</td>
<td>0.32</td>
</tr>
<tr>
<td>Preculmination</td>
<td>0.54</td>
<td>0.55</td>
</tr>
<tr>
<td>Late culmination</td>
<td>0.63</td>
<td>0.53</td>
</tr>
<tr>
<td>Fold increase (amoeba-culmination)</td>
<td>9.4</td>
<td>42</td>
</tr>
</tbody>
</table>

* Enzyme was prepared in 0.01 M Tris (pH 8.5) containing 0.01 M UDPG.

** Enzyme was prepared in 0.01 M Tris (pH 8.5) without UDPG. Cells were ruptured by freezing and thawing, and the supernatant fluid was assayed according to the procedure described in Materials and Methods. The reaction time was 5 min at 23 °C.

TABLE 1. Enzyme specific activity as a function of developmental stage

Development. UDPG has a slight stabilizing effect on the enzyme and was added to the samples that were assayed immediately; complete stabilization could not be achieved. Whether the enzyme was prepared by freezing the cells or rupturing them by passage through a French pressure cell at 30,000 psi, the overall results were the same.

The stability of the enzyme was next examined at five successive stages, from amoeba to preculmination. The data in Fig. 1 clearly indicate a progressive and striking change in enzyme stability as differentiation progressed.

Attempts were then made to obtain clear evidence for the existence of an inhibitor in the extracts prepared from cells in the earlier stages of development. A typical experiment is shown in Fig. 2. Again, extreme instability was observed for the enzyme prepared from amoebae, and complete stability for the enzyme present at preculmination. After inactivation of the amoeba enzyme (30 min at 35 °C), a sample was mixed with the preculmination enzyme. The latter enzyme, which had previously shown complete stability at 35 °C, now followed an inactivation curve similar to that of the enzyme from amoebae. Incubation of the amoeba extract at 100 °C for 5 min destroyed the inhibitor. Figure 2 also demonstrates that a similar kinetics of enzyme inactivation occurs at 25 °C. One curve is plotted on a logarithmic scale (see insert).

DISCUSSION

Since the kinetics of enzyme inactivation are nonlinear, even in the presence of UDPG, it is not possible to predict the specific enzyme activity in the early stages of differentiation. The extent of inactivation between the time of cell rupture and immediate assay can be neither determined nor estimated. Furthermore, had the first assay been performed at 10 min, it would have appeared as though inactivation was linear and as though extrapolation to zero-time was possible. Had it been possible to assay at 1-min intervals during the first 5 min, the inactivation curve may well have been more strikingly nonlinear. After the late aggregation or early pseudoplasmodium stage of development, specific enzyme determinations can be made in the relative absence of artifacts in vitro. The stage at which such artifacts play a minor role can vary from one group of cells to another, depending upon the stage of the cells at the time of harvest or the temperature at which differentiation occurs, or both.

In any event, the significance of changes in enzyme specific activity to reaction rates in vivo
FIG. 2. Amoeba and preculmination enzymes were prepared as described in Materials and Methods after rupture of the cells by freezing. The enzymes were incubated at 35 C (or 25 C as indicated), and samples were removed periodically for assay. A sample of the amoeba extract which was incubated for 30 min at 35 C was then added in a 1:1 ratio to the preculmination extract. Samples comparable to that of preculmination alone were assayed during incubation of the mixture at 35 C. The initial enzyme activity (before incubation) was taken as 100%. The insert shows a logarithmic plot of the lowest curve (amoeba, 35 C).

cannot be predicted. In fact, reactions in vivo have been shown to increase in the absence of changes in enzyme specific activity, as well as to decrease in spite of increases in enzyme concentration (8). The rate of synthesis of UDPG increases about threefold in vivo from the aggregation to the culmination stage of development (4).

It has recently been demonstrated that enhanced enzyme levels cannot be responsible for this increased rate of synthesis, in view of the substrate-$K_m$ ratios involved and the accumulation patterns during differentiation of glucose-1-phosphate and UDPG (Wright, Simon, and Walsh, unpublished data).

ACKNOWLEDGEMENT

This investigation was supported by Public Health Service grant GM15938-01 from the National Institute of General Medical Sciences.

LITERATURE CITED