Cytoplasmic pH and glycolysis in the *Dictyostelium discoideum* cell cycle

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Lactate production measurements during the cell cycle of synchronized populations of *Dictyostelium discoideum* cells reveal cyclic variations in glycolysis which correspond with pH_i oscillations which were discovered by us previously [(1985) Cell, in press]. Aerobic lactate production varies about 6-fold during the cell cycle and the lactate maxima correlate with (~0.25 pH unit) cyclic increases in pH_i. However, artificially altering pH_i using weak acids or bases does not influence the rate of lactate production in asynchronous cell populations. This result suggests that the cyclic variations in pH_i and those in glycolytic rate are not causally related events.

(Dictyostelium discoideum) *pH* *Glycolysis* *Cell cycle*

1. INTRODUCTION

There is growing evidence that intracellular pH (pH_i) may be involved in the regulation of such diverse processes as gamete activation, cellular dormancy, the cell cycle and stimulus-response coupling (review [2]). In connection with this, pH_i is known to influence various biochemical and metabolic processes, including the glycolytic pathway [2,3]. Glycolytic rates increase with increasing pH_i in *E. coli*, Ehrlich ascites cells and red blood cells [4–7]. Stimulation of glycolysis by insulin in frog muscle is also mediated by a rise in pH_i and this response is well characterized [8]. However, the situation is not so straightforward in other cell systems. For example, studies on mitogenically stimulated fibroblasts led to the conclusion that, here, an observed rise in pH_i is not necessary for glycolysis and that there are additional signals which activate glycolysis [9]. Also, in bacterial spores, the rise in pH_i seen at germination is not the sole mediator of the concomitant increase in glycolytic rate [10].

pH_i measurements in synchronized populations of *Dictyostelium discoideum* cells show a clear variation in pH_i during the cell cycle. Furthermore, these pH_i variations coincide with variations in the rates of both DNA and protein synthesis [1]. Here we report that these cyclic alkalinizations also coincide with cyclic increases in the rate of glycolysis. However, attempts to increase glycolytic rate by artificially raising pH_i were unsuccessful, indicating that there is probably not a causal relationship between pH_i and glycolytic rate during the *D. discoideum* cell cycle.

2. MATERIALS AND METHODS

2.1. *Cell culture and determination of lactate production*

Cells of *D. discoideum* (strain Ax-2) were cultured axenically in suspension culture in HL-5 medium at 22°C as described [11].

Aerobic lactate production was determined enzymatically using the Sigma lactate assay kit. At the same time aliquots of the same batch of cells were used for determination of the protein content

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of the cells. This was done by homogenizing the cells in a solution containing Coomassie brilliant blue G-250 and measuring the absorbance at 595 nm with bovine serum albumin as a standard [12]. The rate of lactate production was calculated as the amount of lactate produced (pmol)/h per μg cellular protein.

2.2. *Cytoplasmic pH (pH_i) measurements*

Cytoplasmic pH of *D. discoideum* cells was estimated by means of a digitonin 'null-point' method [13] as described [1]. In short, after harvesting and washing by low-speed centrifugation, the cells were resuspended into 0.35 ml of a weakly buffered potassium phosphate solution (2 mM) at a density of $1-2 \times 10^8$ cells/ml. The cell suspension was continuously stirred and its temperature was maintained at 22°C. The external pH was continuously monitored using a mini pH-electrode, coupled to a pH-meter and a pen recorder and external pH was adjusted to the required value by aliquots of KOH or H_3PO_4. Subsequently, digitonin (0.1%, w/v) was added to the suspension to permeabilize the plasma membranes. The external pH value at which digitonin induced no apparent shift in external pH was taken as an estimate of pH_i. A correction for the background acidification rate (mainly due to stirring in of CO_2) was made in all determinations.

3. RESULTS

3.1. *Physiological alkalinizations parallel increases in glycolytic rate*

Adding digitonin to a *D. discoideum* cell suspension causes a disruption of the plasma membranes of the cells, without affecting the membranes of intracellular organelles such as mitochondria, endoplasmic reticulum and acidic vacuoles [1,14]. So the external pH value at which digitonin addition causes no apparent proton flux into or out of the cells is a good measure of cytoplasmic pH in *D. discoideum*.

Changes in both pH_i, measured by the digitonin-disruption method, and the rate of aerobic lactate production were followed during the cell cycle of synchronized populations of cells. pH_i shows cyclic alkalinizations in the range 7.20–7.45 with time intervals of about 4–5 h [1]. These alkalinizations coincide with cyclic increases in the rate of lactate production as well as with cell division steps (fig.1). Fig.2 illustrates the correlation observed between pH_i and glycolytic rate, namely, when pH_i is high (7.4–7.5), glycolytic rate is also high and when pH_i is low (7.2–7.3), glycolytic rate is low.

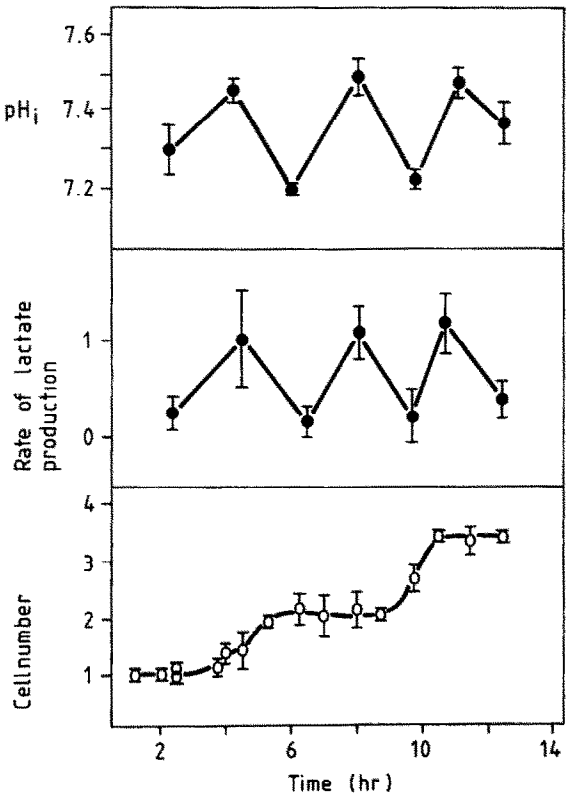

Fig.1. pH_i and lactate production during the cell cycle. Synchronized cell populations were obtained by resuspending stationary-phase cells into fresh medium at a density of about $1-3 \times 10^6$ cells/ml. After this treatment the cells start synchronous cycles of cell division and DNA replication [15,16]. Directly after synchronizing the cells determinations were started at regular 2–2.5 h intervals of both pH_i and the rate of aerobic lactate production (see section 2). The data shown are averaged from 3 experiments. (Top) pH_i (as a function of time), (center) lactate production, (bottom) cell number.

3.2. *Artificially manipulating pH_i does not influence glycolytic rate*

To investigate the possible regulatory influence of pH_i on the rate of glycolysis in *D. discoideum*, we performed a series of experiments in which the

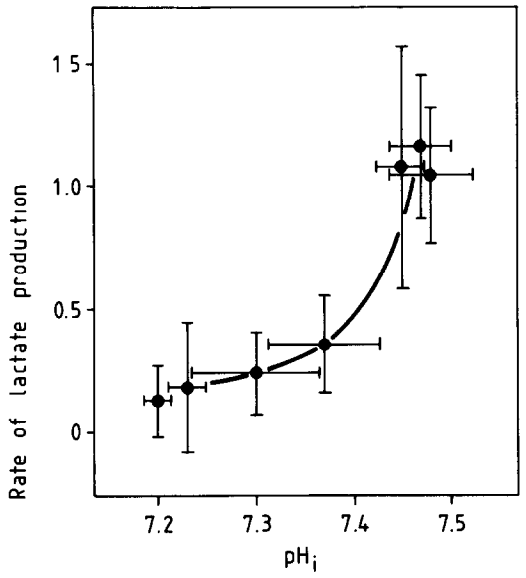

Fig.2. The relationship between pH_i and lactate production during the cell cycle. The data for lactate production and pH_i from fig.1 are plotted against each other. The graph demonstrates a correlation between pH_i and lactate production. If pH_i is high, lactate production is high and if pH_i is low, lactate production is low.

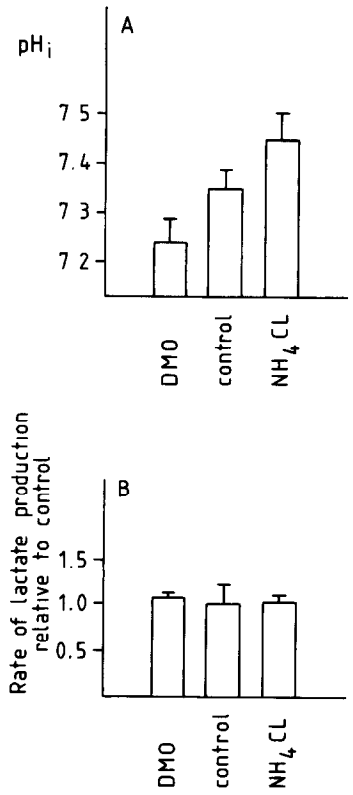

Fig.3. The influence of artificial manipulation of pH_i on lactate production. Asynchronous populations of cells were incubated with ammonia (3 mM at an external pH of about 7.4) or DMO (10 mM at an external pH of about 6.6). After 45 min the influence of these treatments on both pH_i (A) and the rate of lactate production (B) were determined. Means ± SE of 3–4 determinations are shown. The figure shows that manipulating pH_i between about 7.25 and 7.45 has no significant effect on lactate production.

rates of lactate production were compared before and after artificially altering pH_i. To this end, asynchronous cell populations were incubated for 45 min with either ammonia or the metabolically inert weak acid DMO. Fig.3A shows that these treatments obviously alter pH_i; the change in pH_i in both directions is about 0.1 pH unit. However, the rate of glycolysis is not at all influenced by either treatment (fig.3B), indicating that a change in pH_i is not sufficient to bring about a change in glycolytic flux in *D. discoideum*.

4. DISCUSSION

pH_i oscillates during the cell cycle of *D. discoideum* cells and these pH_i oscillations have been reported to coincide with oscillations in the rates of both DNA and protein synthesis [1]. In the same communication experiments were described which revealed that the increase in the rate of DNA and protein synthesis concomitant with the rise in pH_i during the cell cycle can be mimicked by artificially raising the pH_i of asynchronous populations of cells. Here, we report that the cyclic alkalinizations during the cell cycle also coincide with cyclic increases in glycolytic flux (fig.1). However, in marked contrast to the rates of DNA and protein synthesis, the rate of glycolysis cannot be increased simply by raising pH_i in asynchronous populations of cells (fig.3). So, although there is good evidence that pH_i regulates DNA and protein synthesis in *D. discoideum* [1], it does not appear to be an important factor governing glycolytic flux in this organism.

D. discoideum is not the only system in which a rise in pH_i occurring concomitantly with an in-

crease in glycolytic rate turns out not to be of crucial importance for glycolysis. For instance, adding serum or growth factors to quiescent human fibroblasts results in both a rise in pH_i and an increase in aerobic lactate production. Blocking the rise in pH_i, however, does not influence the stimulating effect on glycolysis by serum or growth factors [9]. Likewise, the rise in pH_i that occurs during the germination of bacterial spores is not the most prominent factor influencing the concomitant activation of glycolysis because an artificial alkalinization of dormant spores fails to stimulate glycolytic flux [10]. However, this situation varies considerably between systems and pH_i can have an important role in glycolytic regulation. Thus the stimulation of glycolysis by insulin in frog muscle is apparently due to an accompanying rise in pH_i (review [8]). Here, the stimulation of glycolysis is inhibited if pH_i elevation is blocked, while artificially increasing pH_i in the absence of insulin stimulates the glycolytic flux.

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