MAGNESIUM IONS AND SERUM ACTIVATION OF URIDINE UPTAKE BY QUIESCENT HAMSTER FIBROBLASTS

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1. Introduction

The uptake of uridine by quiescent cells in culture is one of the earliest processes stimulated by the addition of serum [1,2]. It has been shown that the intracellular trapping (phosphorylation) of uridine is activated rather than its transport across the cell membrane [3–5]. Uridine kinase is the enzyme responsible for the first and rate limiting step of uridine trapping [3]. This enzyme requires the presence of Mg^{2+} as its cofactor [6]. Rubin proposed [7] that control of the availability of intracellular Mg^{2+} for transphosphorylation reactions and the synthesis of macromolecules has a key role in the cellular response to stimulation by serum. The effect of Mg^{2+} on the uptake of nutrients by cultures of chick embryo fibroblasts has been demonstrated [8].

McKeehan and Ham [9] have shown that the cellular multiplication rate and cellular survival rate are dependent upon the concentration of divalent metal ions in the growth medium. In this study we show that serum activation of uridine uptake by quiescent NIL 8 hamster fibroblasts is expressed only in the presence of divalent metal ions. $MgCl_2$ stimulates uridine uptake in the presence of serum in a saturable manner. Addition of Mg^{2+} affects both the time course and extent of activation by serum.

2. Experimental

2.1. *Cell culture*

NIL 8 is a clone of golden hamster fibroblasts, and was a gift from the late Dr M. MacPherson of the Imperial Cancer Research Fund, London. The cells were grown and maintained in Dulbecco's modified Eagle's Medium (DMEM) to which 10% newborn calf serum, antibiotics and glutamine (Biolab, Jerusalem) had been added. Cells were propagated from frozen stocks at 6 week intervals. The cells were grown at 37°C in a CO_2-enriched, humidified atmosphere, on 90 mm plastic petri dishes (Nunc Denmark), and were subcultured twice weekly and seeded at ~5×10^5 cells/90 mm dish.

Cells were prepared for uptake experiments in the following manner: NIL 8 cells were subcultured in 35 mm plastic petri dishes at 10^5 cells/dish, the culture medium was replaced 2–3 days after seeding by a low serum medium (0.25% newborn calf serum in DMEM) the cells were ready for the experiment 72–96 h after medium replacement. The cells that undergo the above treatment reach quiescence as judged by the low incorporation of radioactive thymidine into acid-insoluble pools.

2.2. *Reagents*

PBS, phosphate buffered saline (pH 7.4) prepared with double distilled water contained: NaCl, 8 g/l; KCl, 0.2 g/l; $Na_2HPO_4 \cdot 12\ H_2O$, 2.9 g/l; KH_2PO_4, 0.2 g/l.

The dialyzed newborn calf serum (3 days against saline), used in the activation experiments was provided by Biolab Jerusalem.

2.3. *Uptake experiments*

Uptake experiments were performed in 35 mm culture dishes. The culture medium was removed by suction and the cells washed with PBS or with the

solution used for incubation prior to the uptake itself. The uptake of labelled uridine into acid soluble pools was measured as follows: At time zero, 0.5 ml solution of [5-^{3}H]uridine (Israel Nuclear Research Centre, Negev) at 1 μCi/ml in buffer or DMEM was added to each dish. The dishes were kept for the desired period of time in a constant temperature water bath. The uptake was terminated by 5 quick washes with 2 ml of ice-cold PBS and 0.75 ml 5% trichloroacetic acid was added for 20 min at 4°C to extract acid-soluble material. The radioactivity of 0.5 ml aliquots was determined in a liquid scintillation counter.

3. Results

The activation of uridine uptake into quiescent NIL 8 or 3T3 cells, by serum (or insulin) has been studied extensively in this [4,5] and other laboratories [1–3]. Two experimental procedures were applied: the cells were either incubated in DMEM containing the activator prior to the uptake experiment, or exposed to the activator at the same time as the labelled uridine.

The medium, used for cell growth, contains, besides all the essential nutrients and antibiotics, a variety of cations. Since this study was carried out in order to investigate the role of divalent cations in the process of activation, the first aim was to find a proper, well-defined buffer system in which the preincubation as well as uptake experiments could be performed.

Parallel cultures were incubated for 90 min [5] at 37°C in PBS, PBS containing 0.5 mM $MgCl_2$ and 0.7 mM $CaCl_2$, DMEM, and a buffer containing 150 mM NaCl and 30 mM Hepes (pH 7.4). The incubation mixtures contained either 10% dialyzed newborn calf serum or the buffer itself. The incubation was terminated, and labelled uridine solution made up in the same buffer as the activation mixture was added to the cultures (no serum was present in the uptake study) at 25°C. The results of the uridine uptake experiments are shown in fig.1. Two conclusions can be drawn from this experiment:

1. Uridine uptake is faster in the presence of phosphate buffer than in Hepes buffer or DMEM.
2. The presence of divalent metals is necessary for the expression of the effect of dialyzed serum on uridine uptake.

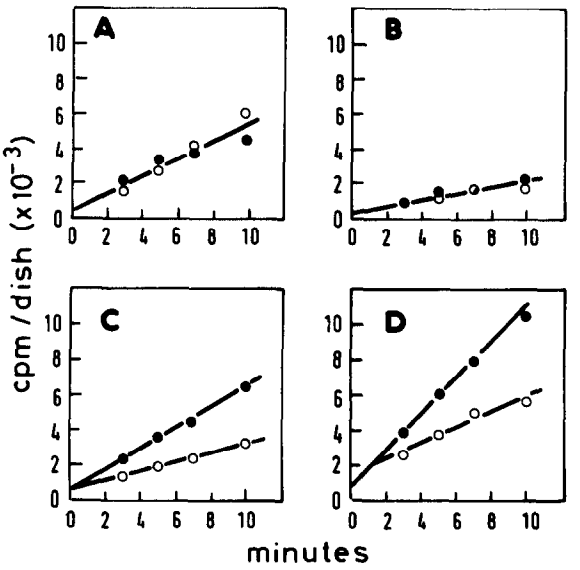

Fig.1. Uptake of uridine into acid soluble pools of NIL 8 cells as a function of time. Quiescent cells were incubated for 90 min at 37°C in various media in the presence (•) or absence (○) of 10% serum. Following this treatment the uptake of 5 μM [^{3}H]uridine was assayed at 25°C. The various media were: A, PBS; B, Hepes (30 mM Hepes (pH 7.4) in 150 mM NaCl); C, DMEM (Dulbecco's Modified Eagles Medium); D, PBS containing 0.5 mM $MgCl_2$ and 0.7 mM $CaCl_2$.

The extent of activation in buffer containing divalent ions is similar to that observed in the growth medium, DMEM.

The second relevant question was whether addition of Mg^{2+} alone can reproduce the conditions in which the uridine uptake system is sensitive to the addition of serum. Experiments identical to the above (fig.1), were carried out in PBS with the addition of various concentrations of $MgCl_2$.

The results of these experiments are depicted in fig.2, where the activation ratio, defined as the ratio between the rate of uridine uptake in presence and in absence of serum, is plotted as a function of Mg^{2+} concentration. It is apparent that serum activation occurs in the presence of >1 mM Mg^{2+} and the extent of activation saturates at about the same level as in DMEM (1.97) and PBS containing both Mg^{2+} and Ca^{2+} (1.8) (see fig.1C,D).

Rozengurt and Stein [2] have studied the time course of the 'switching on' of uridine uptake by serum in 3T3 cells. They found this to be a cooperative

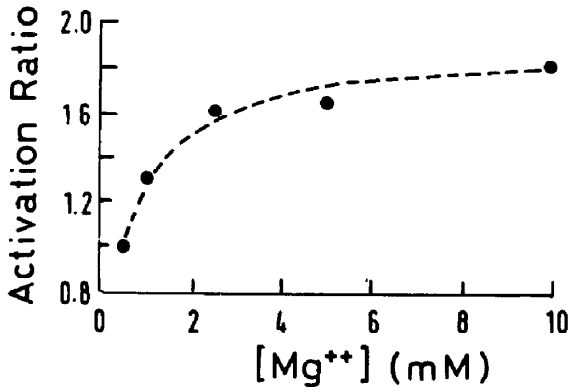

Fig.2. The effect of increasing Mg^{2+} concentration on the extent of activation ratio, defined as the ratio between uridine uptake rate in presence and absence of serum. Quiescent cells were incubated for 90 min at 37°C in PBS containing the appropriate Mg^{2+} concentration in the presence or absence of 10% serum. Incubation was terminated and uridine uptake (5 μM) was assayed for 20 min. Rates were obtained as slopes of uptake curves by linear regression.

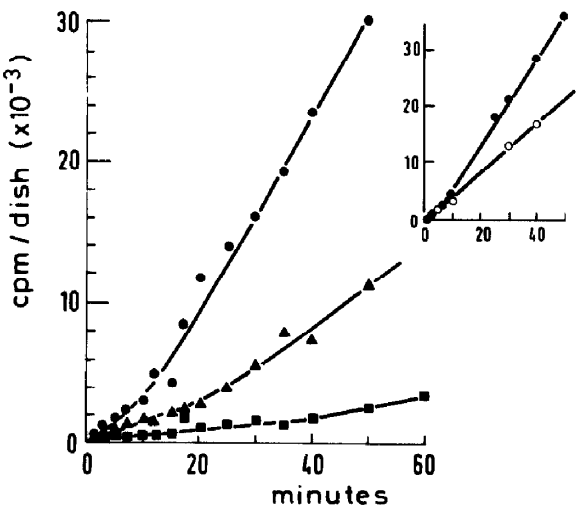

Fig.3. The effect of Mg^{2+} on the process of activation of uridine uptake. A solution of 10% serum in PBS containing Mg^{2+} at: 0 mM (■), 1 mM (▲); 5 mM (●); and 5 μM [^{3}H]uridine was added to quiescent cells at time zero. The accumulation of labelled material was followed as explained in the text. Inset: The uptake of [^{3}H]uridine in the presence of 5 mM Mg^{2+} in absence (○) and in presence (●) of 10% dialyzed serum. Uridine and serum were added at the same time.

process in the sense that it is an 'all or none phenomenon'. The transition from the state of low uridine uptake rate to that of the high rate occurs at a well-defined moment and no state of intermediate rate can be identified. This 'switching on' process can be controlled both by a change in the lag time (before switching occurs) and by a change in the ratio between the unstimulated and final stimulated rate. In order to answer the question as to whether 'lag time' or 'final rate' are affected by $MgCl_2$ the experimental procedure was slightly modified in a similar manner to that in [2]. Labelled uridine is added to the cells at the same time as the serum in the appropriate solution of $MgCl_2$ in PBS, and both uptake and activation occur at the same time in a 37°C water bath. This procedure allows us to follow the time course of the activation process. The results of these experiments are depicted in fig.3. As expected, the incorporation of labelled uridine into acid-soluble pools is not linear with time for the whole duration of the experiment. At the initial phase of the experiment the uptake rate is low, typical for the unstimulated state. In a control experiment (inset to fig.3) it has been established that the initial uridine uptake rate in the presence of serum is identical to the rate in the absence of serum. The uptake of uridine is essentially linear with time until the 'lag time' is over, at that point a sharp transition to the high, stimulated rate occurs, and the uptake is again linear with time. The intersection point between the 'early' and 'late' linear components of the uridine uptake curve in the presence of serum is referred to as 'lag time'. Two conclusions can be drawn from the experiments described in fig.3:

1. 90 min, which was the preincubation time for both experiments described above, is much longer than the lag time under all experimental conditions. Thus, the effect of Mg^{2+} is on the final extent of activation by serum. Although the experimental conditions are slightly different, the conclusions drawn from fig.1,2 are verified here. Comparison of the final uptake slopes of fig.3 reveals a strong stimulating effect by added $MgCl_2$. This effect is even more pronounced than in the uptake rates after long incubation time (compare to fig.2). This difference may be due to the fact that here the uptake is measured at 37°C and not at 25°C. A slight effect of increasing Mg^{2+} concentration on the unstimulated uptake rate is

observed here, but it is clear that in the presence of serum the effect is much more significant.

2. An obvious relationship between lag time and Mg^{2+} concentration is apparent. The lag time is reduced from 20–10 min when Mg^{2+} concentration is increased from 1–5 mM. It seems that the rate limiting step of the action of serum requires the presence of the divalent metal.

It has been shown [5] that during the first 30 s of uridine uptake by NIL 8 cells, transport across the plasma membrane is the rate limiting step. We have measured the rates of uridine transport (at 5 μM) under some of the conditions described above, namely, in the presence of 0, 1 and 10 mM Mg^{2+}, after 90 min pretreatment with serum, in order to check whether this initial phase of uptake is also affected by Mg^{2+}. The rates of transport were: 14 ± 4; 14 ± 1 and 10 ± 2 pmol $.min^{-1}$ $.dish^{-1}$ for 0, 1 and 10 mM Mg^{2+}, respectively. It is apparent that transport is not enhanced by Mg^{2+} under the conditions where significant effects are observed at long uptake times. One may conclude therefore that the effects described in the present study occur at the second phase of the uptake process, namely, phosphorylation of uridine.

4. Conclusions

Serum stimulation of uridine uptake by quiescent NIL 8 cells occurs only in the presence of divalent cations. Mg^{2+} affects the rate, as well as the extent of activation by serum, and the step susceptible to this effect is the phosphorylation of uridine within the cell rather than its transport into it.

Our results are consistent with the assumption that uridine uptake stimulation is coupled to the activation of a carrier protein which is responsible for the enhanced transport of Mg^{2+} across the plasma membrane. This model is in line with the findings in [10] that the intracellular concentration of free Mg^{2+} increases upon the addition of insulin to quiescent chick embryo fibroblasts.

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References

[1] Jimenez de Asua, L., Rozengurt, E. and Dulbecco, R. (1974) Proc. Natl. Acad. Sci. USA 71, 96–98.
[2] Rozengurt, E. and Stein, W. D. (1977) Biochim. Biophys. Acta 464, 417–432.
[3] Rozengurt, E., Stein, W. D. and Wigglesworth, M. (1977) Nature 267, 442–444.
[4] Koren, R., Shohami, E., Bibi, O. and Stein, W. D. (1978) FEBS Lett. 86, 71–75.
[5] Heichal, O., Ish-Shalom, D., Koren, R. and Stein, W. D. (1979) Biochim. Biophys. Acta 551, 169–186.
[6] Anderson, E. P. (1973) The Enzymes 9, 49–64.
[7] Rubin, H. (1975) Proc. Natl. Acad. Sci. USA 72, 3551–3555.
[8] Bowen-Pope, D. F. and Rubin, H. (1977) Proc. Natl. Acad. Sci. USA 74, 1585–1589.
[9] McKeehan, W. L. and Ham, R. G. (1978) Nature 275, 756–758.
[10] Sanui, H. and Rubin, H. (1978) J. Cell. Physiol. 96, 265–278.