FOLIC ACID INCREASES THE cAMP BINDING ACTIVITY OF *DICTYOSTELIUM DISCOIDEUM* CELLS

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1. Introduction

Starvation triggers the development of *Dictyostelium discoideum* cells. During the preaggregation and aggregation stages, a number of biochemical changes occur in the cells [1]. One of the most important events is the production and the subsequent pulsatile emission of adenosine 3′5′-cyclic monophosphate (cAMP) which has been known in this organism to play various roles in cell differentiation as well as in cell aggregation [1,2]. For example, pulse additions of exogenous cAMP to cells in the early developmental stage induce biochemical changes in the cells [3], including that of the activity of adenylate cyclase [4,5] and phosphodiesterase [5–7]. These effects of cAMP must primarily be mediated by cAMP receptors located on the cell surface [8–11].

On the other hand, folic acid which is known as a chemoattractant for cells in the early developmental stage [12,13] induces biochemical oscillations, stimulates the development of the cells [14] and increases intracellular cGMP level [15,16] in *Dictyostelium*. This suggests that folic acid plays an important role in cell differentiation, and hence the question arises as to whether folic acid has any role in cAMP-mediated biochemical changes.

Here it is shown that folic acid increases cAMP binding activity of *D. discoideum* cells, suggesting that folic acid facilitates the cells to respond to extracellular cAMP.

2. Materials and methods

Dictyostelium discoideum NC-4 was used. Amoebae were grown in association with *Escherichia coli* B/r, on a nutrient agar [17]. To obtain cells in various developmental stages, washed growing-stage cells were incubated on a Millipore filter saturated with 20 mM MES buffer (pH 6.0) at 21°C. In a folic acid pulse experiment, washed growing-stage cells were suspended in 20 mM MES (pH 6.0), at $1–3 \times 10^7$ cells/ml, and incubated at 21°C on a gyratory shaker (120 rev./min). After 2.5 h incubation, the cell suspension was pulsed with 8×10^{-7} M folic acid (final conc.) 7–8 times at intervals of 8 min.

To assay cAMP binding, cells were washed twice with cold 20 mM MES buffer (pH 6.0), once with the same buffer containing 10 mM dithiothreitol (DTT) [9]. Unless indicated otherwise, the cells were incubated for 2 min at 5°C with 40 nM or 220 nM c[^{3}H]-AMP, 10 mM DTT and with or without folic acid in test tubes. The binding which takes place within 1 min was directly assayed with the cell suspension placed in centrifuge tubes. Duplicate samples were placed on a silicone layer (ρ = 1.01–1.03) over 15% sucrose solution containing 1% SDS, and then centrifuged for 30 s in a Beckman Microfuge B. The cytosols in the bottom layer were dissolved in PCS (Amersham). Non-specific binding was determined by performing the binding assay with an excess of non-radioactive cAMP and was subtracted from the total binding. Each experiment represents the results from 2 independent examinations.

3. Results

Whether or not folic acid increases the cAMP binding activity of cells was studied. Washed growing-stage cells were allowed to differentiate, for indicated hours, on a Millipore filter as in section 2. Cyclic

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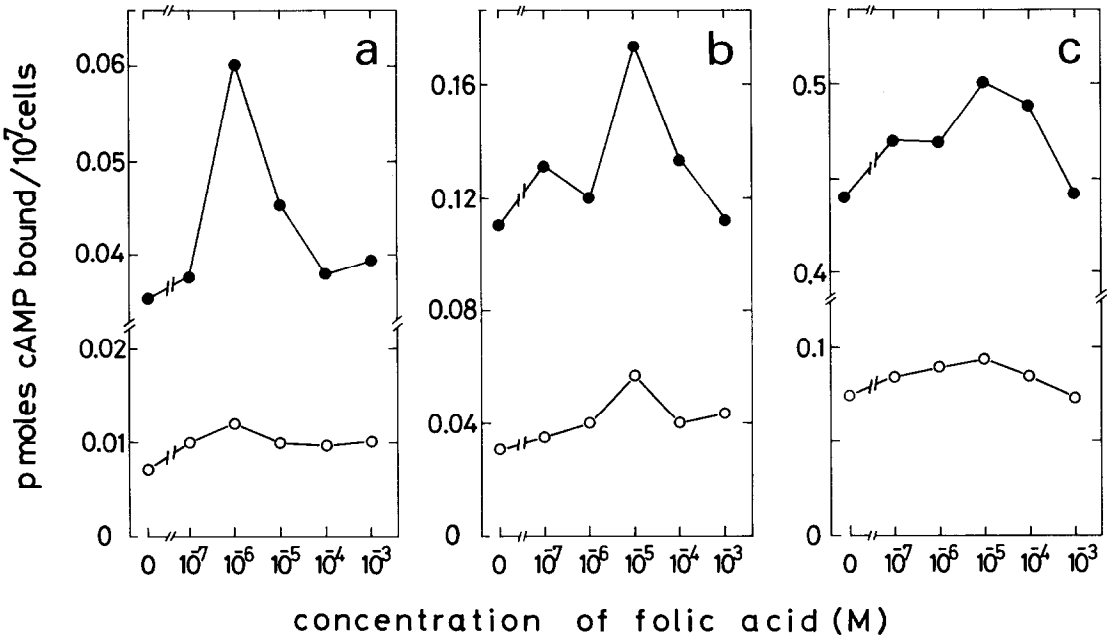

Fig.1. Effect of folic acid on cAMP binding to cells in various developmental stages. Cyclic AMP binding to the cells which had been incubated for (a) 0.5, (b) 3 and (c) 7.5 h on a Millipore filter was measured, in the presence of (—○—) 40 nM or (—●—) 220 nM c[^{3}H]AMP and with 0–10^{-3} M folic acid, for 2 min at 5°C, respectively.

AMP binding activity of cells was measured for 2 min at 5°C, with 40 or 220 nM c[^{3}H]AMP, and with folic acid within 0–10^{-3} M. The NC-4 preaggregation cells have apparently 2 binding sites (high affinity 7 nM and low affinity 180 nM, respectively) (S. K., unpublished). As shown in fig.1, in any developmental stages of cells, folic acid at $\geq 10^{-7}$ M increased the cAMP binding to the cells as measured either with high or low concentrations of c[^{3}H]AMP; with cells starved for 0.5 h the maximum cAMP binding was observed at 10^{-6} M folic acid, while in cells starved for 3 and 7.5 h the maximum binding was at 10^{-5} M folic acid. These results suggest that folic acid increases the number of cAMP molecules bound to the cells.

At 10^{-6} M folic acid, cAMP binding to cells starved for 0.5 h showed that the amounts of cell-bound c[^{3}H]AMP reached a plateau after 15 s incubation and remained at this level for ≥5 min (fig.2). The same binding time-course was observed in the cells without the addition of folic acid (data not shown). No significant difference in the cAMP binding activity of the cells was found when the binding to cells starved for 3 h was measured for 2 min at 5,10,15,20 and 25°C, respectively (data not shown). The same tem-

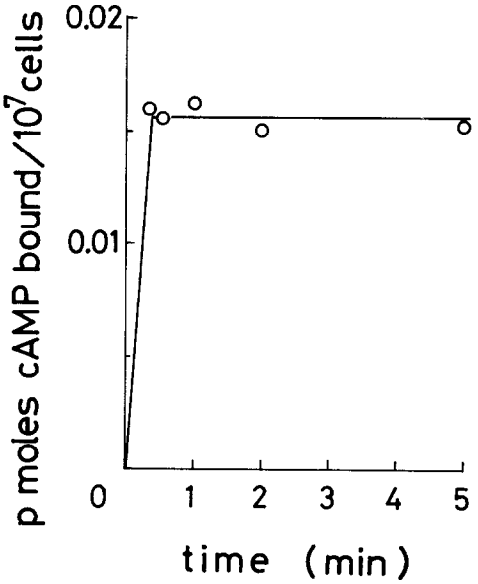

Fig.2. Time course of c[^{3}H]AMP binding to cells starved for 0.5 h. Cyclic AMP binding to the cells was measured, in the presence of 40 nM c[^{3}H]AMP and 10^{-6} M folic acid, for the times indicated, at 5°C.

perature independence of the binding was also observed in the cells without folic acid. Hereafter, the cAMP binding, with or without folic acid, was determined after incubation for 2 min at 5°C.

Since in preaggregation cells, the cAMP binding activity of the cells markedly increases in the presence of low concentrations of $CaCl_2$ [18]. The effect of Ca^{2+} on the cAMP binding of cells starved for 5 h in the presence of folic acid was studied. As shown in fig.3, the cAMP binding to the cells markedly increased upon addition of 5 mM $CaCl_2$, whereas a further increase in the binding was observed when the cells were incubated with 5 mM $CaCl_2$ plus folic acid; the maximum binding was observed at 5 mM $CaCl_2$ plus 10^{-5} M folic acid. These results indicate that folic acid increases the cAMP binding activity of the cells independently of Ca^{2+}.

Whether or not cAMP binding activity of cells is stimulated by pulse additions of folic acid was studied. Cell suspension which had been incubated for 2.5 h in 20 mM MES buffer (pH 6.0) was pulsed with 8×10^{-7} M folic acid (final conc.) 7–8 times at intervals of 8 min. Then, the cells were twice washed and

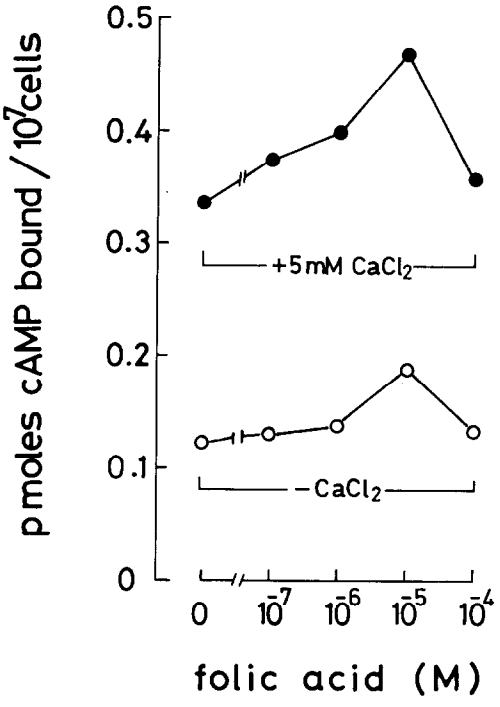

Fig.3. Effect of Ca^{2+} on cAMP binding to cells. Cyclic AMP binding to cells starved for 5 h was measured, with or without 5 mM $CaCl_2$ and with $0-10^{-4}$ M folic acid, for 2 min at 5°C.

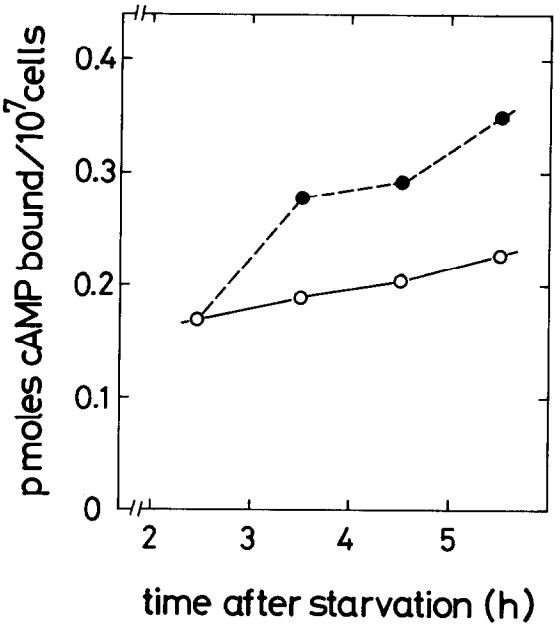

Fig.4. Effect of folic acid pulses on cAMP binding to cells. Cells starved for 2.5 h in 20 mM MES buffer (pH 6.0) were pulsed with 8×10^{-7} M folic acid (final conc.) 7–8 times, with a period of 8 min. After pulsed, the cells were twice washed, and cAMP binding to the pulsed cells (–•–) and to the non-pulsed cells (–○–), respectively, was measured with 220 nM c[^{3}H]AMP, without folic acid, for 2 min at 5°C.

their cAMP binding activity was measured without folic acid. As shown in fig.4, the binding of the pulsed cells was greater than that of non-pulsed cells. This increase in the binding of the pulsed cells was maintained during further incubation (fig.4). On the other hand, after a single addition of 2×10^{-5} M folic acid to cells starved for 2.5 h, cAMP binding of the cells, as measured after 15 min, increased by 100% over the control. Then, however, it decreased to the control level as measured after 1 h incubation. These results indicate that pulse additions of folic acid more effectively stimulate the cAMP binding activity of cells than a single addition of folic acid.

4. Discussion

Folic acid, a chemoattractant of *D. discoideum* cells [12,13], induces oscillations of the intracellular cAMP level [14] and increases the intracellular cGMP level [15,16]. Here, I have attempted to establish whether or not folic acid plays any role in the cAMP-

mediated biochemical changes. A possibility that folic acid stimulates the adenylate cyclase which is activated by cAMP [4,5,19] has been discussed [14], however, no direct evidence has been presented to support this idea. Also, no significant activation of guanylate cyclase coupled with the folic acid-induced increase in the intracellular cGMP level was found [20], whereas cAMP directly stimulates the enzyme [21].

This study shows that folic acid increases the amount of cAMP bound to the cells and that pulse additions of folic aicd stimulate the cAMP binding activity of the cells. These facts show that folic acid increases the sensitivity of the cells to extracellular cAMP. When folic acid is added to the cells in adequate pulses, it stimulates the differentiation of the cells as a result of repeated activation of their sensitivity to cAMP.

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