S-ADENOSYLMETHIONINE INHIBITS Ca^{2+} UPTAKE INTO *ESCHERICHIA COLI* MEMBRANE VESICLES

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1. Introduction

We have recently observed that the addition of *S*-adenosylmethionine (SAM) inhibits ATP-dependent $^{45}Ca^{2+}$ uptake into *Dictyostelium discoideum* vesicles (J. M. M., M. Cao, in preparation). Whereas nothing is known about the function of SAM in *Dictyostelium*, there is a well established relationship between protein methylation and the processing of chemotactic stimuli in bacteria [1]. Further, inverted membrane vesicles isolated from bacteria are known to accumulate $^{45}Ca^{2+}$ in the presence of ATP [2,3]. Therefore, we decided to examine the effect of SAM on $^{45}Ca^{2+}$ uptake into *Escherichia coli* vesicles. We show that 1 mM SAM inhibits by ~7-fold the uptake of $^{45}Ca^{2+}$ into *E. coli* vesicles prepared by lysis with a French pressure cell.

2. Methods

2.1. *Assay of $^{45}Ca^{2+}$ transport*

Transport of $^{45}Ca^{2+}$ was essentially measured as in [2]. *E. coli* BB/13 (10 g) were suspended in a buffer containing 10 mM Tris–HCl (pH 7.2); 0.14 M KCl, 1.2 mM 2-mercaptoethanol and 10% (v/v) glycerol (TKMG buffer). The cells were then lysed by 2 passes through a French pressure cell (Aminco) at 3000–4000 lb/in^2. Each step of this procedure was performed at 4°C and the homogenate was kept on ice until use. The homogenate was diluted 3 times in 10 mM Tris–HCl (pH 8.5) at 22°C and after incubation for 30 s the transport of $^{45}Ca^{2+}$ was carried out at 22°C in a 1100 μl reaction volume containing 10 mM Tris–HCl, (pH 8.5), 0.14 M KCl, 5 mM $MgCl_2$, 1 mM ATP, 10 μM $^{45}Ca^{2+}$ (0.1 μCi ^{45}Ca/ml, Amersham) 2 mM potassium phosphate (pH 8.5) and 110 μl homogenate (~1 mg protein). At various times 200 μl aliquots were added to 900 μl terminating ice-cold buffer containing 10 mM Tris–HCl (pH 8.5), 0.14 M KCl and 10 mM $CaCl_2$. One ml was then filtered on 24 mm filters (0.45 μm pore size, Schleicher and Schüll) and washed twice with 3 ml terminating solution. The radioactivity of the filters was then measured by liquid scintillation counting. Non-specific $^{45}Ca^{2+}$ binding was measured by carrying out the assay in the presence of a 1000-fold excess of $CaCl_2$.

2.2. *Preparation of membrane vesicles pre-loaded with $^{45}Ca^{2+}$*

In another series of experiments, after dilution of the cells lysed in 10 mM Tris–HCl (pH 8.5) the transport of $^{45}Ca^{2+}$ was carried out at 22°C in 5 ml reaction volume with the same composition as the above procedure. After a 2 min incubation, 25 ml ice-cold terminating buffer was added and the mixture centrifuged 2 h at 48 000 × *g* (at 4°C in a SS-34 Sorvall rotor). The pellet was resuspended with a Dounce homogeniser in 8 ml terminating buffer and centrifuged again at 48 000 × *g* for 2 h at 4°C. The washed pellet was then resuspended with a Dounce homogeniser in 2 ml of the same buffer at 4°C and three 100 μl portions of the mixture filtered on 0.45 μm filters and after washing twice with 3 ml terminating buffer the radioactivity of the filters was measured. Protein content was determined by the

method in [5]. ATP and SAM were purchased from Boehringer.

3. Results

Accumulation of $^{45}Ca^{2+}$ was found to occur in homogenates of *E. coli* prepared by lysis with a French pressure cell. In the absence of ATP the uptake of $^{45}Ca^{2+}$ was 20–30% of that found in its presence. At 1 mM, SAM inhibited by ~6-fold the accumulation of $^{45}Ca^{2+}$ (fig.1a). SAM at 0.1 mM inhibited by only 10–20% and 0.01 mM SAM had no effect on the uptake of $^{45}Ca^{2+}$. When 1 mM SAM was added to a cell homogenate pre-loaded with $^{45}Ca^{2+}$ for 1 min, it induced a fast release of radioactivity into the incubation medium (fig.1b). In another series of experiments, lysed *E. coli* cells were allowed to accumulate $^{45}Ca^{2+}$ for 2 min in the presence or absence of 1 mM SAM. Then, membrane vesicles were isolated by centrifugation as in section 2 and the amount of $^{45}Ca^{2+}$ retained measured. Up to 7-fold more $^{45}Ca^{2+}$ was found in the particles incubated in the absence of SAM (table 1). Transport of $^{45}Ca^{2+}$ was also measured in membrane vesicles which were isolated without previous incubation with $^{45}Ca^{2+}$. In the presence of ATP the transport of $^{45}Ca^{2+}$ by these vesicles was similar in the absence or presence of 1 mM SAM (table 2).

Table 1
Uptake of $^{45}Ca^{2+}$ into *E. coli* membrane vesicles

Addition	^{45}Ca cpm/mg protein
None	5940
1 mM SAM	840

See section 2 for a description of the procedure

Table 2
Uptake of $^{45}Ca^{2+}$ into *E. coli* membrane vesicles

Addition	^{45}Ca cpm/mg protein
None	5920
1 mM SAM	6800

After lysis 5 ml *E. coli* homogenate was diluted with 25 ml ice-cold TKMG buffer and centrifuged 2 h at 48 000 × *g* at 4°C. The pellet was resuspended in 8 ml TKMG buffer and centrifuged again at 48 000 × *g* for 2 h at 4°C. The pellet was resuspended in 2 ml TKMG buffer, diluted 3 times in 10 mM Tris–HCl (pH 8.5) at 22°C and the transport of $^{45}Ca^{2+}$ measured for 2 min as in section 2

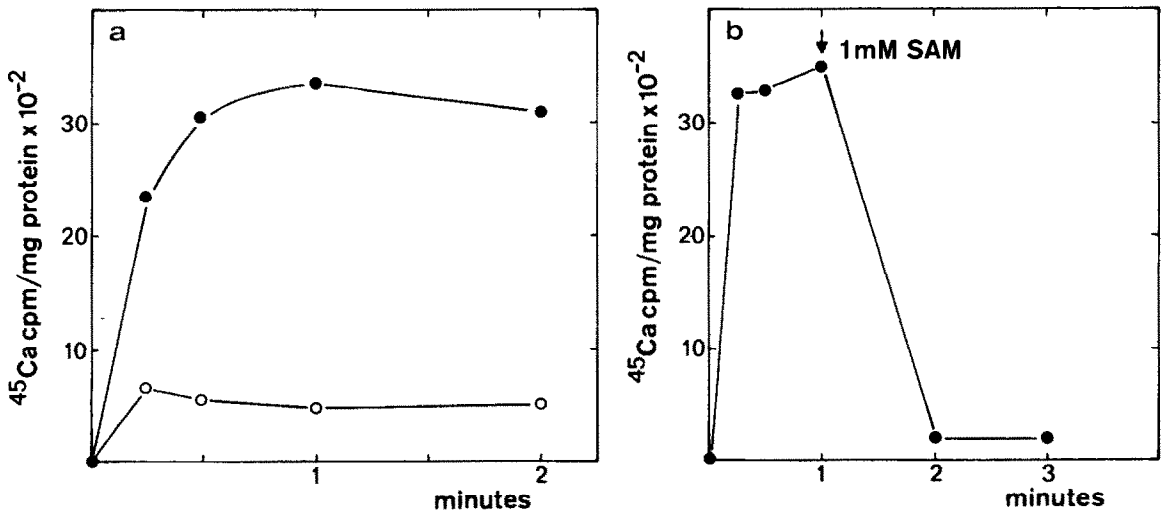

Fig.1. Effect of SAM on $^{45}Ca^{2+}$ uptake by *E. coli* homogenates prepared by lysis with a French pressure cell. In a, the uptake of $^{45}Ca^{2+}$ was assayed in the absence (•) or presence (○) of 1 mM SAM. In b, $^{45}Ca^{2+}$ was allowed to accumulate during 1 min as in section 2. Immediately after the removal of the 1 min sample, SAM (final conc. 1 mM) was added and 1 and 2 min later samples were withdrawn and the $^{45}Ca^{2+}$ uptake assayed.

4. Discussion

Bacteria vesicles prepared by lysis with a French pressure cell are believed to be inverted membrane vesicles [2,3]. Such vesicles accumulate $^{45}Ca^{2+}$ in the presence of ATP and the results in vitro might reflect the mechanism that is in vivo responsible for the active efflux of Ca^{2+} from bacteria cells [2,3]. The present results show that SAM inhibits the uptake of $^{45}Ca^{2+}$ into *E. coli* vesicles. Because the methylase is a cytoplasmic enzyme, incubations were performed with the crude homogenate rather than with the isolated vesicles. As shown in table 2, isolated *E. coli* vesicles transported $^{45}Ca^{2+}$ but the addition of SAM had no effect on the accumulation of $^{45}Ca^{2+}$. Whether SAM inhibits Ca^{2+} uptake by making vesicles more permeable to Ca^{2+} or by an inhibition of Ca^{2+} transport is not known. In both cases SAM would induce an increase in intracellular Ca^{2+} by either increasing the diffusion of Ca^{2+} from the external medium or by inhibition of its efflux.

Bacteria swim smoothly in a straight line for ~1 s, then tumble for < 1 s, then swim again in a random direction [5]. When exposed to a sudden increase of attractant tumbling is suppressed and after an adaptation period the normal tumbling frequency is observed again [5]. Methionine is required for the transition from smooth swimming to tumbling, that is for adaptation to increases in the concentration of stimulus [7–9]. The methionine requirement reflects a need for SAM. Chemotactic stimulation has been shown [1] to influence the methylation of an inner membrane protein of about 60 000 daltons (MCP). Evidence which indicates that above a certain cytosolic Ca^{2+} level bacteria tumble continuously is given in [10]. The present results indicate that the function of SAM in vivo might be to increase the intracellular Ca^{2+} in *E. coli*. If so, the increase in methylation observed during chemotactic stimulation may induce a transient increase in intracellular Ca^{2+} which will restore a normal tumbling frequency. According to the model in [11] for chemotaxis in bacteria, in the presence of a stimulus the levels of an unknown compound, x, increases but must recover to its steady state level soon after stimulation. Thus, a possible function of Ca^{2+} during chemotactic stimulation in bacteria might be to activate the enzyme responsible for restoring the concentration of x to its pre-stimulation steady state level. The fact that SAM also inhibits $^{45}Ca^{2+}$ uptake into *D. discoideum* vesicles (J. M. M., M. Cao, in preparation) suggests a basic mechanism for this molecule in controlling cellular Ca^{2+} movements.

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