MANGANESE ACCUMULATION BY ESCHERICHIA COLI: EVIDENCE FOR A SPECIFIC TRANSPORT SYSTEM

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Summary - Manganese-54 is accumulated by cells of Escherichia coli by a process which is temperature dependent and inhibited by dinitrophenol and by cyanide. The rate of uptake of manganese is concentration dependent with a K_m of about 5×10^{-8} M in dilute tryptone broth. The ^{54}Mn which is accumulated by the cells is not tightly bound since it can be released by toluene and exchanges rapidly with excess nonradioactive manganese. The manganese accumulation system is highly specific: potassium, strontium, calcium and magnesium do not compete with manganese for cellular uptake.

Manganese-54 is accumulated by cells of Escherichia coli by an active transport system which appears to be distinct from other bacterial active transport mechanisms. Some of the basic properties of this system are shown in the experiment in Figure 1:

(i) Manganese-54 is accumulated against a large concentration gradient. The cells, which occupy about 0.1 % of the culture volume, can accumulate more than 50 % of the added ^{54}Mn, which makes a concentration gradient of about 1000:1 (assuming that the cellular volume is about 75 % water and that the accumulated manganese is free and not bound).

(ii) More than 80 % of the accumulated ^{54}Mn is relatively free within the cells and not tightly bound, as judged by its rapid release upon the addition of toluene (Fig. 1) and rapid exchange with extracellular manganese (Fig. 2). Toluene does not lyse the cells but simply makes them leaky to small molecules (1,2). The release of manganese after toluene treatment is more rapid than is the release of radioactive magnesium (3) or potassium (unpublished data) from the cells under similar conditions.

(iii) The accumulation of ^{54}Mn is partially inhibited by energy poisons such

as sodium cyanide and dinitrophenol (DNP) (Fig. 1). Different active transport systems show different degrees of inhibition by cyanide and DNP and the level of inhibition is also dependent upon the growth medium (unpublished data). The manganese system in dilute broth is the least sensitive to cyanide inhibition of the four (magnesium, manganese, potassium, and thiomethylgalactoside) which have been studied in this laboratory. Cyanide at 1 mM inhibits the uptake of radioactive manganese by 50 to 80 % (in different experiments) whereas 1 mM cyanide inhibits potassium accumulation by more than 99 %. DNP at 1 mM inhibits the rate of manganese accumulation more completely than cyanide--the inhibition ranged from 85 % (Fig. 1) to more than 98 % (Fig. 2) in five independent experiments.

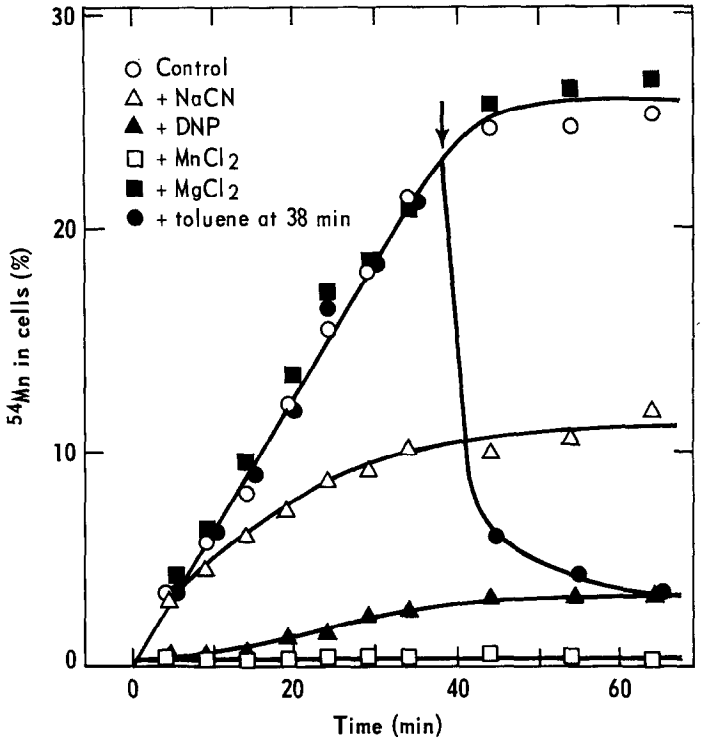

Fig. 1. Accumulation of ^{54}Mn by E. coli: specificity, inhibition and release by toluene. E. coli strain B was grown at 37° C in dilute tryptone broth (4 g/l Bacto-tryptone and 2.5 g/l NaCl) to a density of 1.0 x 10^9 cells/ml. The culture was divided into aliquots and cooled to 26° C. Two minutes prior to the addition of 0.25 μc/ml ^{54}Mn (10^{-9} M; New England Nuclear), 1 mM NaCN, DNP, $MgCl_2$ or $MnCl_2$ was added to four aliquots. Toluene (1% v/v) was added to another aliquot at 38 minutes. Samples were removed, filtered through Millipore HA filters, washed with 10 ml broth, and counted in a Nuclear Chicago gas-flow counter with approximately 1 % efficiency (cpm/dpm).

(iv) The manganese accumulation system in E. coli is highly specific. The addition of 1 mM $MgCl_2$ to the cells two minutes before the addition of ^{54}Mn is without effect on manganese uptake (Fig. 1), but the addition of 0.01 mM $MnCl_2$ (Fig. 2) or 1 mM $MnCl_2$ (Fig. 1) reduced the accumulation of radioactivity at least 100-fold. KCl, $SrCl_2$, $MgCl_2$ and $CaCl_2$, when added in separate experiments at 5 mM, were without striking effect on ^{54}Mn uptake in dilute broth. By varying the concentration of nonradioactive $MnCl_2$ added prior to ^{54}Mn and by reducing the specific activity of the carrier-free ^{54}Mn by mixing it with nonradioactive manganese, we have obtained a "K_m" (4) for the manganese accumulation system of approximately 5 x 10^{-8} M.[1] The K_m for the magnesium active transport system in tryptone broth is about 5 x 10^{-4} M (3).

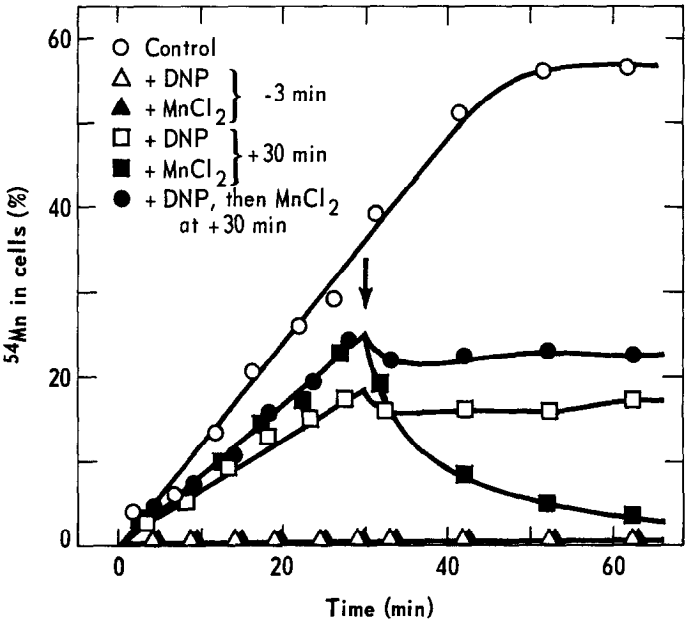

Fig. 2. DNP inhibition of manganese turnover. DNP (1 mM) or $MnCl_2$ (0.01 mM) was added either 3 minutes prior to or 30 minutes after the addition of 0.2 μc/ml ^{54}Mn to 5 x 10^8 cells/ml at 26° C. To one sample, first DNP and then $MnCl_2$ 15 seconds later were added 30 minutes after the addition of ^{54}Mn.

[1] The value of 5 x 10^{-8} M is actually an upper limit for the K_m. We do not know the manganese content of the dilute tryptone broth (below the detectible limits of atomic absorption spectroscopy) which could increase the apparent K_m by as much as a factor of two. The K_m for manganese accumulation in phosphate-buffered minimal medium is several orders of magnitude higher (unpublished data).

There are important properties which are useful in establishing the existence of active transport systems in addition to those demonstrated in Figure 1. These include: (i) the swelling and shrinking of osmotically fragile forms in response to changes in the external concentration of a substance (which is generally considered evidence that the internal material is osmotically free and not tightly bound), and (ii) the coupling of efflux with influx (which shows that exit is not by leakage but carrier mediated (4)). We cannot demonstrate osmotic swelling and shrinking when the K_m is less than 10^{-7} M and the osmotic strength of our dilute broth is about 0.1 Osm. At concentrations about 10^{-7} M even if all of the manganese is accumulated by the cells and osmotically active, it will represent only a very small fraction of the species the cells utilize to maintain osmotic equilibrium. The coupling of manganese efflux to influx, however, is demonstrated by the following experiment. DNP inhibits manganese uptake; if exit is coupled to entrance, DNP should prevent the chasing of radioactive manganese from the cells by excess nonradioactive manganese. This is what is seen in Figure 2. When nonradioactive 0.01 mM $MnCl_2$ is added to the cells 30 minutes after the addition of 0.001 μM ^{54}Mn, there is a rapid loss of more than 80 % of the ^{54}Mn from the cells as nonradioactive manganese replaces radioactive manganese. If, however, DNP is added just prior to the addition of nonradioactive manganese, it completely inhibits the chase (Fig. 2).

Active transport systems generally show a greater dependency of uptake on temperature than do those of passive transport or binding. In Figure 3, the cellular accumulation of ^{54}Mn (actively accumulated) is compared with cellular accumulation of ^{45}Ca (passive binding). Over the temperature range from 25° C to 45° C, the accumulation of ^{45}Ca in a 2 minute exposure does not discernibly change. However, the accumulation of ^{54}Mn increases 7.5-fold between 25° C and 45° C. As the incubation temperature is raised above 50° C, the accumulation of manganese drops rapidly as the cells are thermally killed, but the accumulation of ^{45}Ca increases 5-fold on thermal killing. More calcium

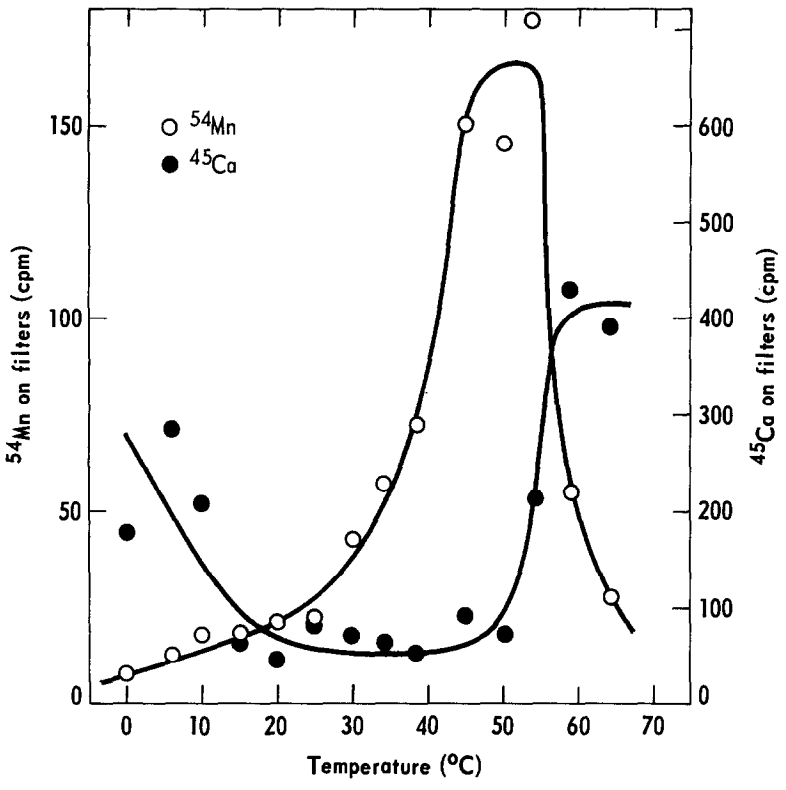

Fig. 3. Temperature dependence of ^{54}Mn and ^{45}Ca accumulation by E. coli. Broth-grown cells were centrifuged and concentrated. The cells were then diluted 25-fold to 5.5 x 10^8/ml into tryptone broth with added ^{54}Mn (0.2 μc/ml) or ^{45}Ca (0.25 μc/ml) which had been temperature-equilibrated in one of fourteen water baths. Samples were filtered and washed (with iced, room temperature or heated broth) after 45, 90 or 120 seconds. The accumulation of manganese increased with time; that of calcium was not time dependent. Only the 2-minute data are shown. 100 cpm ^{54}Mn represents 3.3 % uptake and less than 3 x 10^{-3} μmoles/10^{12} cells; 100 cpm ^{45}Ca represents 0.05 % uptake, but 9 x 10^{-2} μmoles/10^{12} cells.

is also accumulated at very low temperatures (Fig. 3).[2]

This is a first report of a specific manganese accumulation system in bacterial cells. More detailed reports of the requirements for manganese uptake and of the intracellular distribution of manganese must await further

[2]We think that the increased uptake of calcium at low and high temperatures compared with the uptake at physiologically normal temperatures for E. coli is a consequence of a metabolically active system for the extrusion or pumping of calcium out from the cells (5,6). The data in Figure 3 are consistent with but do not establish the existence of such a calcium transport system.

work. Because of interactions between the magnesium and manganese contents of bacterial cells (3,7,8), we had expected that manganese would be transported into the cells by the magnesium active transport system. This did not turn out to be the case. In mitochondria, calcium and manganese appear to share an active transport system (9). The experiments reported here, however, exclude this possibility in E. coli cells. Manganese can play many intracellular roles including activation of RNA polymerase (10,11) and cell wall synthesizing enzymes (12,13). Indeed manganese stimulates the growth of magnesium-limited bacteria (8). Perhaps the more efficient activation of some enzymatic activities by manganese when compared with magnesium (10-13) has provided the selective pressure for the evolution of a specific manganese accumulation system with an unusually low K_m.

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