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SHORT COMMUNICATIONS

Influence of diethylenetriamine pentaacetic acid (DTPA) on the synthesis of DNA, RNA, and protein in *Escherichia coli*

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Chelating agents are used for therapy of metal intoxications in humans [1]. In recent years compounds such as DTPA (diethylenetriamine-pentaacetate) have been reported to be compounds of choice especially for the decorporation of radioactive ions [2]. DTPA binds metal ions with different affinity, complex stability increasing in the order Mg < Ca < Mn < Zn. Although the administration of Ca-salts reduced efficiently the toxicity of these agents, toxic side effects are still restricting their use in medicine. These effects are connected with an inhibition of DNA [3–5], RNA and protein syntheses [6]. Administration of Zn^{2+} and Mn^{2+} ions abolished toxic side effects and normalized DNA synthesis.

With bacteria, the chelating agent DTPA has not been tested. Only Na-EDTA (Versene) has been studied. After brief treatment at concentrations in the m-mole range it increases membrane permeability in gram-negative bacteria [7–10] by the removal of calcium which seems correlated to the loss of lipopolysaccharide (LPS) from the outer membrane. The selective release from Salmonella cells of ATPase, malate dehydrogenase and acid phosphatase has been reported [11].

In the study reported here we present results on the influence of Ca-DTPA and Zn-DTPA on DNA, RNA, and protein metabolisms in growing *Escherichia coli*. We found kinetics similar to the action of Na-EDTA.

The experiments were performed with *Escherichia coli* CR34 which requires threonine, leucine, and thymine for growth, is lactose-negative, and is T1 and T5 resistant. Methyl-[^{3}H]-thymine (23 mCi/m-mole) and 5-[^{3}H]uridine (5 Ci/m-mole) were obtained from the Radiochemical Centre Amersham, England. [^{14}C]-1-Leucine (312 mCi/m-mole) was bought from Schwarz–Mann Biochemicals, USA. To prepare the Ca- and Zn-chelates of DTPA (Fluka, Switzerland) equimolar amounts of $CaCl_2$ and ZnO, respectively, were mixed with H_5DTPA and NaOH in water. The pH was adjusted to 7.3 with NaOH.

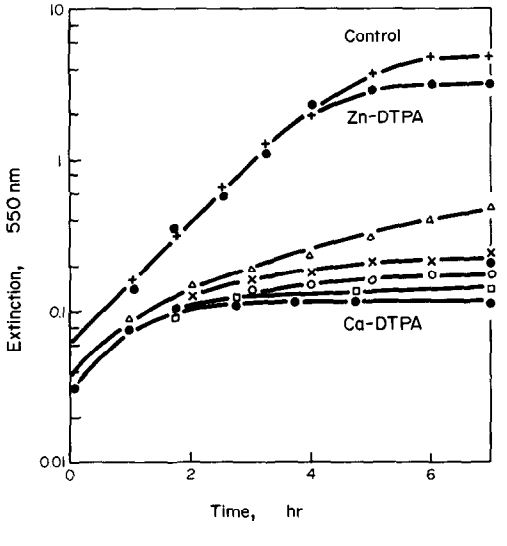

Fig. 1. Influence of Ca-DTPA and Zn-DTPA, respectively, as a function of concentration on the growth of *E. coli* CR34 as measured by absorption at 550 nm.

One ml (titer 2×10^8 cells/ml) of a log-phase culture of *E. coli* CR34 in M9 medium supplemented with 10^{-2} M Ca^{2+} and caseinhydrolysate, was centrifuged and the sediment resuspended in 10 ml of the same medium. The final specific radioactivity was 150 μCi/m-mole[^{3}H]thymine, 1.5 mCi/m-mole[^{3}H]uridine and 54 μCi/m-mole[^{14}C]leucine, respectively. The cells were grown with aeration at 37°. Samples of 0.1 ml were withdrawn at fixed time intervals and mixed with 3.9 ml of 5% trichloroacetic acid (TCA). After precipitation at 0° for 10 min, each sample was filtered through a Sartorius membrane filter (0.45 μm) and washed with 75 ml boiling distilled water containing 25 μg leucine, 50 μg thymine or 50 μg uridine per ml, respectively. The filters were dried and counted in a scintillation fluid [1 l. toluene + 4 g PPO + 0.1 g POPOP (Packard Instr.)], using a Tri-Carb (model 3380) liquid scintillation spectrometer (Packard Instruments Co., U.S.A.).

No matter whether cell growth, cell multiplication, DNA, RNA or protein synthesis were used as experimental end points, the chelating agents exerted similar characteristic effects (Figs. 1 and 2): It took considerable lengths of time, i.e. about 60 min of growth, until the decrease of cell growth or macromolecule synthesis was measurable. While 10^{-6} M still had no effect, an increase to 2.5×10^{-5} M effectively blocked cell growth and macromolecule synthesis. In between this range of concentration a continuously decreasing effect was observed. The effective range of Ca-DTPA concentration was lower by 3 orders of magnitude than the effective concentration of Zn-DTPA, i.e. 1.5×10^{-5} M Ca-DTPA corresponds in its blocking power to 1.5×10^{-2} M Zn-DTPA (Fig. 3). Addition of Zn or Mn reversed the inhibitory action of Ca-DTPA. The difference in delay of metabolic inhibition by Ca-DTPA observed in our experiments with *E. coli* (i.e. 60 min for DNA and protein syntheses, respectively, 40 min for RNA synthesis) is in qualitative agreement with data from a recent work on enzyme activity in red blood cells which is blocked by Ca-DTPA after several hours delay [12].

It is generally assumed that DTPA molecules do not enter the cell. For our experimental results with *E. coli* cells, two alternative explanations are considered:

(i) At concentrations greater than about 2.5×10^{-5} M Ca-DTPA in the cell environment, the metal content of Zn^{2+} and Mn^{2+} inside the cell is reduced. This effect can be interpreted simply as a "leaking out" of metal by diffusion, which is essentially bound to biomolecules, e.g. enzymes, inside the cell. The addition of Zn^{2+} and Mn^{2+}, respectively, to the medium results in a rapid uptake of these elements by the cell and reconstitution of metal–biomolecule complexes. It is also possible that enzyme precursors formed in the absence of the trace metals are being activated after addition of Zn^{2+} and/or Mn^{2+}.

(ii) Two differences of our system compared with Na-EDTA have to be considered. Ca^{2+} cannot be the primary target since Ca-DTPA was used in our experiments and

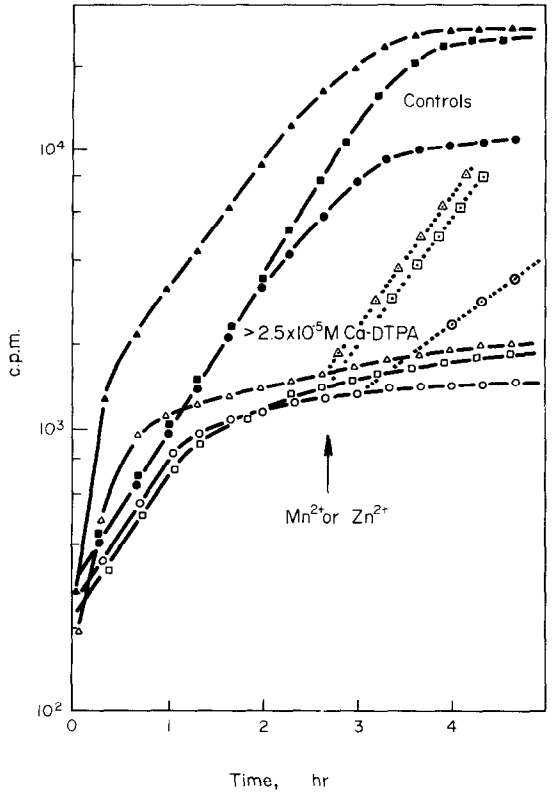

Fig. 2. Influence of Ca-DTPA on DNA, protein and RNA metabolism in *E. coli* CR34 measured by the incorporation of radioactivity (c.p.m.) from [^{3}H]thymine (○, ●, ×), [^{14}C]leucine (□, ■) and [^{3}H]uridine (△, ▲). The arrow indicates the addition of equimolar concentration of Zn^{2+} and Mn^{2+}, respectively, to Ca-DTPA treated cells. ⊙, ▽, △ = incorporation in the presence of the metal ions. (Experimental points give the mean value from at least 5 independent experimental sets.) c.p.m. = radioactive counts in TCA-precipitable material.

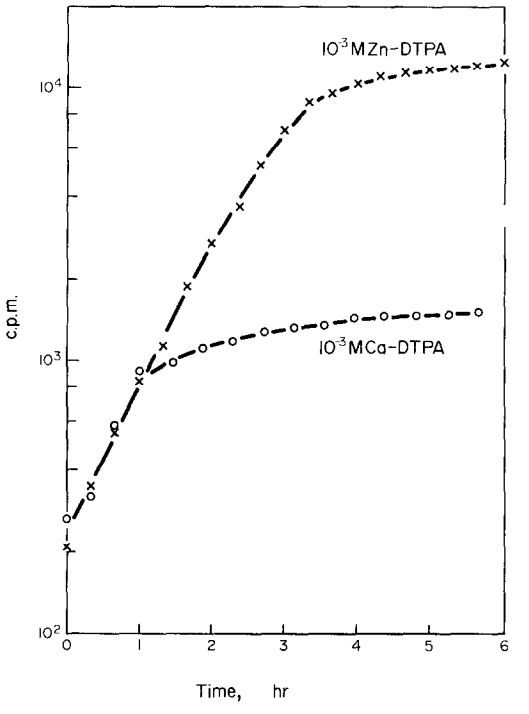

Fig. 3. Comparison of the influence of Zn-DTPA and Ca-DTPA, respectively, at equimolar concentrations on the incorporation of [^{3}H]thymine in *E. coli* CR34.

10^{-2} M Ca^{2+} was added to the growth medium. The influence of Ca-DTPA on metabolism, however, is observed at a concentration as low as 2.5×10^{-5} M. It is the removal of other essential metal ions, such as Zn^{2+} and Mn^{2+}, which apparently causes the effect. In addition to various low molecular compounds, ATP and other triphosphates leak out after EDTA treatment [10, 11]. Leakage of ATP from the cells also occurred with Ca-DTPA in our experiments. Cells were incubated with 2.5×10^{-5} M Ca-DTPA until the complete metabolic block was reached. After ultrasonic disruption ATP content was compared with control cells by the luciferase technique. Ca-DTPA treated cells contained only 20 per cent of the normal ATP concentration measured (3.27 μg ATP/mg protein). This should be due to release of ATP from the cells into the growth medium. Indeed, after sedimentation of cells while had been kept for 5 hr in medium containing 2.5×10^{-5} M Ca-DTPA, the supernatant contained about 50 times more ATP than the control. This could well result in a stop of RNA, protein and DNA synthesis. Addition of Zn^{2+} and/or Mn^{2+} neutralizes the action of the chelating agent. The lag period before the metabolic block is fully expressed and the lag period needed for recovery from Ca-DTPA inhibition is explained by the time needed for releasing and new syntheses of biological compounds, respectively (*cf.* Fig. 2).

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