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Note

INSIGHT ON THE $^{204}Tl^{+}$ – *ESCHERICHIA COLI* SYSTEM

Dan Tiberiu COSTIN, Robert Vasile GRĂDINARU, Gheorghe NEMȚOI and Karin POPA*

"Al.I. Cuza" University, Faculty of Chemistry, 11 - Carol I Blvd., 700506 - Iaşi, Roumania

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Three strains of *Escherichia coli* were studied in order to discriminate between the chemotoxicity and the radiotoxicity of the thallous ion. It was proved that 1 mM Tl^{+} is responsible of a 10-15 % (w/w) decreasing of the *E. coli* cells density, while the $^{204}Tl^{+}$ pure β-emitting isotope can decrease the optical density up to 55 % (w/w) than compared with the reference cell cultures. The chemotoxicity of the thallous ion on *E. coli* is a threshold effect, linearly increasing up to 5 mM Tl^{+} in the growing media and remaining constant for higher concentrations. The microbial *in vivo* reduction of Tl^{+} to Tl^{0} was demonstrated by using cyclic voltammetry.

Even if the interaction between radionuclides and microorganisms was extensively studied during the last decades,[1] the broadness of this area makes the subject still of interest. Microbial processes have an important role in defining the speciation and mobility of heavy metals and radionuclides,[2] phenomena which must be controlled in the nuclear waste repositories. The mobility of radionuclides is also of interest in terms of radiological hazard.[3-5]

Genetically engineered *Escherichia coli* have proved to be a very powerful microbial bioaccumulator for a number of divalent or trivalent cations like Pb^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, and Cr^{3+}.[6-9] By using ^{204}Tl pure β-emitting isotope, it was demonstrated that the thallous ion accumulation in *E. coli* occurs through the potassium transport system.[10] The interference of Li^{+} and Na^{+} on Tl^{+} accumulation by *E. coli* was also reported.[11] The main conclusion of the above mentioned studies is that the heavy metal cytotoxic manifestation may either be mediated through changes in the plasma membrane permeability or efflux of intracellular potassium during the entry of Tl^{+}. Since this presumption does not fully explain the observed redox behavior of the thallous ion, we conducted the present study, which also helps to discriminate between the chemotoxicity and the radiotoxicity of the thallous ion.

RESULTS AND DISCUSSION

The chemotoxic effect of Tl^{+} together with the radiotoxic effect of $^{204}Tl^{+}$ pure β-emitting radioisotope on the three different strains of *E. coli* (the first used mainly for DNA isolation, the second and third for protein expression) are presented in **Fig. 1**.

The reference represents the cell density of *E coli* culture. Since DH5α is a strain used for cloning its optical density is higher in comparison with the other two strains (used for protein production in the host *E. coli*).

It is evident than the chemotoxic effect of the thallous ion is responsible of a 10-15 % (w/w) decreasing of the *E. coli* cells density. This effect is primary due to the surface accumulation of the positive Tl^{+} ion on the negatively charged reactive sites on the biomass surface[12] and to the passive transport of Tl^{+} through the plasma membrane.[13] But having in mind the *in vivo* experimental condition as well as the relative long contact time (14 h), an active accumulation mechanism of Tl^{+}

* Corresponding author: tel.: +40-232-201316, fax: +40-232-201313, e-mail: kpopa@uaic.ro

via either of two Kdp and TrkA K^+ transport systems could be envisaged.[10,11] The chemotoxicity of the thallous ion on *E. coli* is a threshold effect, as demonstrated by incubation of DH5α *E. coli* strain (**Fig. 2**) with different concentrations of Tl^+ overnight. It could be observed that the chemotoxic effect is linearly increasing up to 5 mM Tl^+ in the growing media and then it remains constant. This concentration is 40 times lower than the intracellular concentration of K^+ one. A similar chemotoxic effect cumulated with an inhibitory action on pyruvate kinase I was reported by Umeda *et al.* (1984).[14]

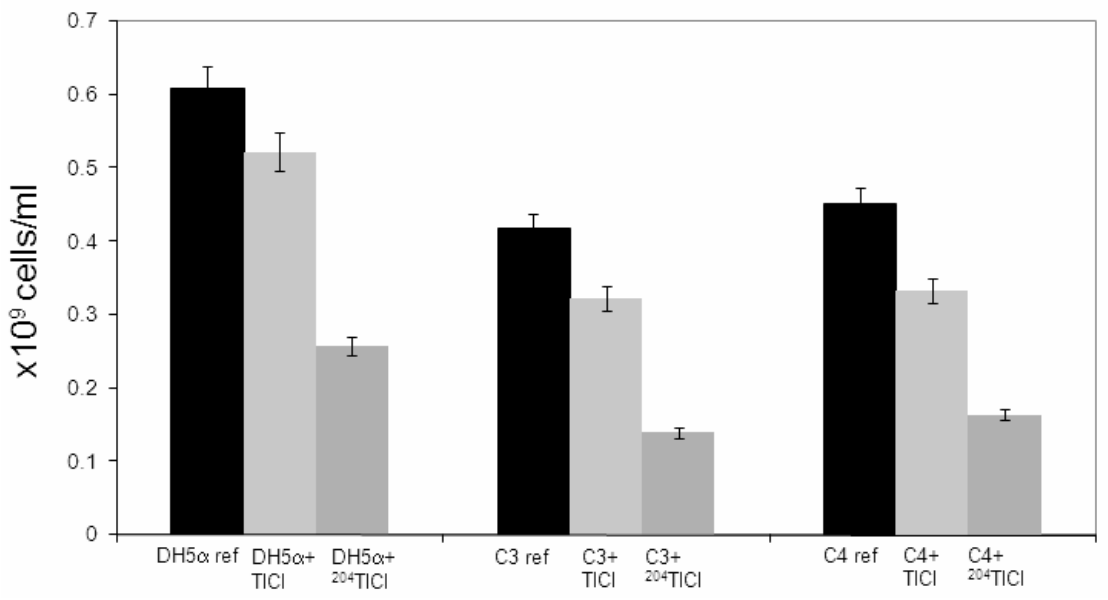

Fig. 1 – Inhibition of *E. coli* cells growth in the presence of 1 mM Tl^+/ $^{204}Tl^+$ as a function of the culture type.

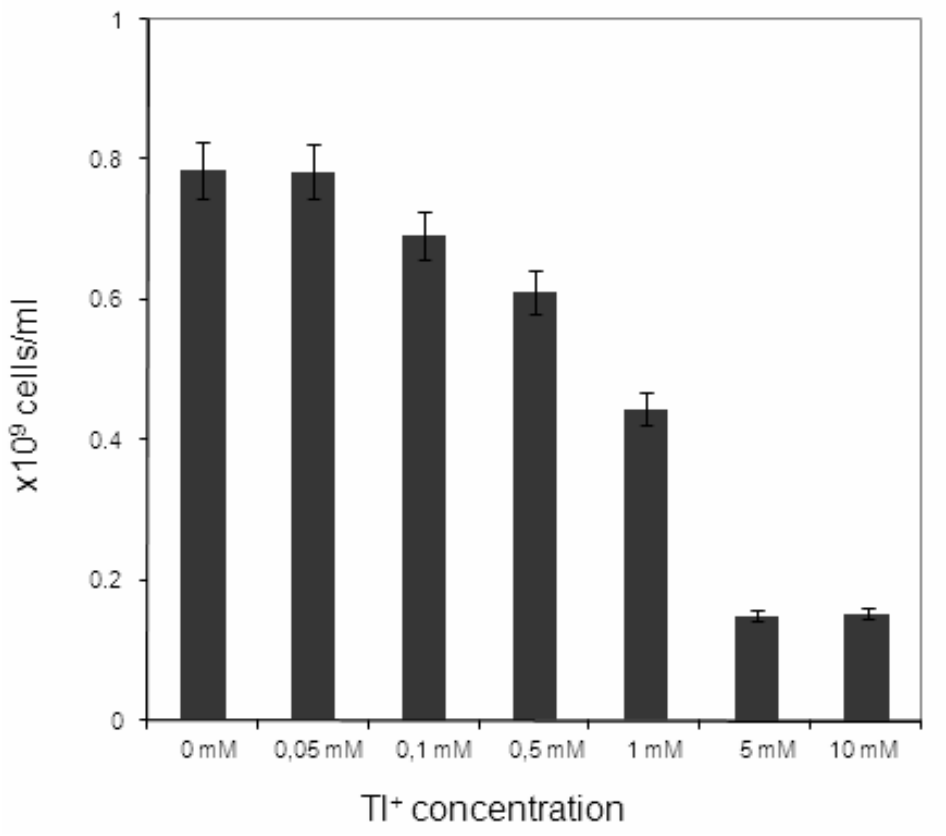

Fig. 2 – Inhibition of DH5α *E. coli* cells growth in the presence of different concentrations of Tl^+.

Once transported inside the living cells, Tl^+ can be released by K^+ addition to the assay medium,[10] which is not the case of our experiment. Thus, once entered in the leaving cells, the ^{204}Tl pure β-emitting isotope (97.1 % β^-, 2.9 % e.c.; half-life 3.78 y) acts as an internal radiation source, damaging the biological material through ionization and excitation mechanisms.[15] This can conduct to an additional radiotoxic effect which can decrease the optical density up to 55 % (w/w) than compare with the reference cell cultures.

The reduction of Tl^+ to the metallic Tl^0 was a slow process observed during the experiments (black powder precipitated on the biological material surface) and proved by cyclic voltammetry (**Fig. 3**).

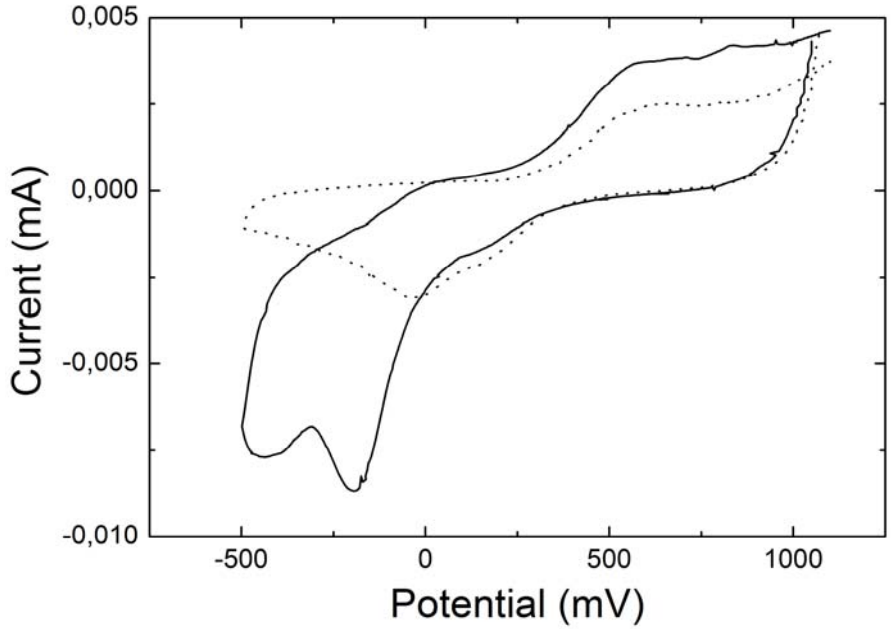

Fig. 3 – Cyclic voltammograms of DH5α *E. coli* system immediately after Tl^+ addition (dot line) and after 3 hours contact time (solid line). Working conditions: domain from 1100 to -500 mV, speed of 50 mV/s, using solutions of LB medium containing 1 mM Tl^+.

After addition of TlCl in the cells medium, a cathodic peak appears at -130 mV with an intensity of 3.23 μA, corresponding to the reduction of the free Tl^+ to metallic Tl, has arisen. The intensity of this cathodic peak is increasing in time, being accompanied by a displacement towards more negative values. Thus, after 1.5 h the potential reach -263 mV for a peak intensity of 6.48 μA; this fact demonstrates that more Tl^+ was reduced. After 3 h contact time, this peak is accompanied by a second one at -410 mV, which is attributed to the reduction of the biologically immobilized Tl^+, in agreement with the results of the previous experiments.[16] On the anodic branch, this second peak is accompanied by an oxidation shoulder which can be attributed to the total reversibility of the Tl^+ reduction. No other electrochemical change was observed between 3 and 24 h contact time in the DH5α *E. coli* - thallium system.

EXPERIMENTAL

Three strains of *E. coli* (further called DH5α, C_3 = C3016, C_4= C2566 from a T7 Express Sampler, New England, BioLabs) were used in our experiments. Precultures of *E. coli* were incubated at 37° C with continuous stirring (100 rpm), in liquid LB medium. In order to differentiate between the chemotoxic and radiotoxic actions of thallous ion on *E. coli* we conducting the following experiment. Triplicates of samples of 1 ml of cell preculture and 19 ml of medium containing 1 mM TlCl (Sigma-Aldrich, 99.9%)/ $^{204}TlCl$ (IFIN-IHH, Măgurele/ Bucharest; the specific activity of the initial $^{204}Tl^+$ solution was 1024 Bq/ml) were incubated at 37° C under stirring for 20 hours. The cells density was determined by reading the absorbance at 580 nm using a Libra UV/Vis Spectrophotometer (Biochrom). The residual activity of the solution was measured by using a

Triather liquid scintillation counter. For calibration, a source of ^{90}Sr of 37000 Bq was employed.

The influence of TlCl concentration was determined as follows. A rich medium culture, prepared by dissolving 20 g LB powder (Roth) in 1 l distilled water, was used and then the medium was sterilized in an autoclave. The stock concentrated TlCl solution was prepared by dissolution of 0.476 g TlCl in 250 ml LB medium (final concentration 10 mM) and filtrated through a CE-membrane (pores size 0.45 μm, Roth) for sterilization. A preculture of DH5α was incubated overnight (14-16 h) at 37° C with stirring using a IKA KS 4000 ic control shaker until an optimal cell density. Triplicate cultures were inoculated by adding 1 ml of *E. coli* preculture in 19 ml LB medium at different final concentrations (10.0, 5.0, 1.0, 0.5, 0.1, and 0.05 mM) of TlCl. These samples were incubated at 37° C for 14 hours, after which the absorbance was measured using an UV-VIS spectrophotometer as described above.

The redox process was studied by using a Voltalab 32 (Radiometric - Copenhagen) electrochemical system which consists of a potentiostat DEA-332 and a digital electromechanical analyzer DEA-1 with a measuring cell with three electrodes. The working platinum electrode was in a shape of a 2 mm diameter disk, while the platinum counter electrode was in the shape of a stick of 1 mm diameter × 10 mm high; the as reference, the saturated calomel electrode was used. The working conditions and procedures are described in a previous work.[16] In short, the cyclic voltammograms were drown between 1100 and -500 mV with a speed of 50 mV/s, using solutions of LB medium containing 1 mM Tl^+.

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