Protection by phytin against Cu\(^{2+}\) ion toxicity in few bacteria

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Individually phytin, EDTA and copper (Cu\(^{2+}\)) have inhibitory effect on growth of Gram positive as well as Gram negative bacteria. Cu\(^{2+}\) has been found to be more toxic to Rhizobium of the four species of bacteria studied. Toxicity of Cu\(^{2+}\) is reduced by EDTA. Differential effect of phytin has been observed between Gram positive and Gram negative bacteria. Phytin preferentially protects only Gram negative bacteria from Cu\(^{2+}\) ion toxicity. This has been interpreted that strong electrostatic repulsion between anionic phytin and anionic biopolymer teichoic acid on the surface of Gram positive bacteria results in inhibition of penetration of phytin into them. Thus the chelating ability of phytin, an anionic biomolecule present in many foodstuff, has some beneficial effect too.

Studies with phytin (Na-salt of phytic acid) have long been of great interest to the scientists for its diverse activity\(^1\)\(^3\). In avian species it has been reported to be beneficial in reducing oxygen affinity of haemoglobin, just like 2,3-diphosphoglycerate in human. It is reported to reduce effectively the proliferation of cancer cells in mammalian intestine\(^4\)\(^5\) and to repress oxidation of lipids\(^6\)\(^7\). Most of the beneficial effects are due to metal chelating ability of phytin\(^8\)\(^9\). Due to its chelating ability phytin reduces the bioavailability of metals when used with foodstuff\(^10\)\(^11\).

Growth of many bacteria is greatly inhibited by heavy metals like copper, mercury, cadmium etc\(^12\). Entering the cell, copper is known to form complex with some chromatophore in Streptomyces pilosus\(^13\). The purpose of this study was to check whether the chelating effect of phytin can be extended to prokaryotic cells also. Two Gram positive bacteria and two Gram negative bacteria were selected for the study. Effect of EDTA, a known metal chelator, was checked to compare with the effect of phytin.

The microorganisms for the study, two Gram negative bacteria, E. coli and a Rhizobium sp. isolated from root nodules of a leguminous herb (Melilotus alba) and two Gram positive bacteria, Bacillus subtilis and Staphylococcus aureus, were obtained from the collection of culture of the Microbiology Section, Department of Botany, Burdwan University, Burdwan, India. Rhizobium sp. was grown in a yeast extract mannitol medium of Skerman\(^14\) with little modification (one litre of medium of pH 7 contained mannitol, 10 g; K\(_2\)HPO\(_4\), 0.5 g; MgSO\(_4\).7H\(_2\)O, 0.2 g; CaCl\(_2\).2H\(_2\)O, 0.1 g and yeast extract, 0.5 g). The other bacteria were grown in nutrient broth at pH 7 (one litre of the medium contained peptone, 5 g; beef extract, 5 g; and NaCl, 5 g). CuSO\(_4\).5H\(_2\)O was used as the source of Cu\(^{2+}\).

To make the inoculum, a loopful of bacteria were grown in 20 ml of medium in a conical flask (100 ml) for overnight on a rotary shaker at 30\(^\circ\)±2\(^\circ\)C. One ml of the inoculum was added to each experimental set containing 20 ml of medium in a conical flask (100 ml) and the bacteria were allowed to grow for 16 hr on a rotary shaker (160 rpm) to get the bacteria at the log-phase of growth. The growth of bacteria was measured turbidimetrically by a colorimeter at 540 nm.

Initially the minimum inhibitory concentration (MIC) of phytin, EDTA and Cu\(^{2+}\) was obtained by growing the bacteria in their respective medium with different concentrations of the chemicals for
overnight. Non-inhibitory concentrations of phytin and EDTA were selected for rest of the experiments. To check the effects of phytin and EDTA on Cu$^{2+}$ toxicity, the bacteria were grown with little less than 50% inhibitory concentration of CuSO$_4$.5H$_2$O for different periods (30 min to 120 min) to allow Cu$^{2+}$ to enter into the cell. Then EDTA or phytin was added to cells and allowed to grow for 20 hr on the shaker. The growth of the bacteria was measured turbidimetrically. The control sets did not contain Cu$^{2+}$.

Growth of all the bacteria was inhibited by Cu$^{2+}$, EDTA and phytin (Table 1). Cu$^{2+}$ was more toxic to E. coli as compared to phytin and EDTA (Fig. 1). Effect of phytin and EDTA was similar. The toxicity of Cu$^{2+}$ was maximum to Rhizobium sp. followed by S. aureus (Table 1). Cu$^{2+}$ is known to be an essential nutrient and also a toxic heavy metal for most living cells\textsuperscript{15}. EDTA and phytin being metal chelators\textsuperscript{2,8,9} are expected to inhibit growth of bacteria.

On treatment with EDTA, inhibition of growth by Cu$^{2+}$ of all the bacteria was arrested (Table 2). There was a differential effect of phytin on Gram positive and negative bacteria for protection (Fig. 2). Inhibition of growth of Gram positive bacteria (B. subtilis and S. aureus) by Cu$^{2+}$ could not be arrested by phytin, but was arrested by phytin in Gram negative bacteria (Table 2). It seems that phytin chelated the metal and thus reduced its toxic effect in Gram negative bacteria. The difference in wall character of two group of bacteria might have some role on this differential effect. Anionic polyl phosphate teichoic acid is present under the surface of Gram positive bacteria\textsuperscript{16,17} and not in Gram negative bacteria. Possibly anionic phytin, with as many as twelve anionic sites per ring of six carbon atoms, suffered a strong electrostatic repulsion from teichoic acid present under the surface of Gram positive bacteria which inhibited the penetration of phytin. At physiological pH, all the four carboxyl groups of EDTA are not fully ionised and there might be some residual intramolecular chelation involving the unionised carboxylic group(s), thus weakening the electrostatic repulsion from teichoic acid. Gram negative bacteria do not normally contain teichoic acid, thus phytin as well as EDTA can penetrate their cell walls.

Effects of phytin and EDTA in reducing the toxicity of Cu$^{2+}$ could be observed if those chemicals were allowed to act within one hour of treatment with Cu$^{2+}$. After one hour the reduction of the toxic effect of Cu$^{2+}$ was much less (Table 2). Cu$^{2+}$ is known to form complex slowly with some cell components\textsuperscript{15}; probably it remained as free ion (Cu$^{2+}$) immediately after entering the cell, when it could be chelated by phytin or EDTA. A part of Cu$^{2+}$ became unavailable to phytin or EDTA when those were used after one hour. The toxic free Cu$^{2+}$ ions that remained inside
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References

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