

to be around 1.7 billion years ago and thereafter. It is inferred that copper was not utilized in the organisms that developed earlier than that time. Most of the copper proteins and enzymes of today are found only in eukaryotes, the more advanced form of organisms. Such proteins include hemocyanin, tyrosine, ceruloplasmin, ascorbate oxidase, laccase and dopamine-beta-hydroxylase. Azurin is found in some aerobic pro-karyotes, whereas plastocyanin and cytochrome c oxidase (copper-dependent) are found in a limited number of cyanobacteria and aerobic bacteria, in addition to most eukaryotes. Superoxide dismutase (SOD) in prokaryotes and mitochondria contain either iron or manganese, while SOD in the cytoplasm of most eukaryotic cells is a Cu,Zn enzyme. It may then be inferred that these organisms, some prokaryotes and most eukaryotes, that contain Cu-dependent proteins could have evolved later than 1.7 billion years ago.

The biological evolution is now studied (among other things) from sequencing of homologous proteins and polynucleotides of different organisms. The information obtained from bioinorganic studies of different elements (such as outlined here) could be complementary to the molecular evolutionary studies in elucidating the biological evolution.

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S7

Protective and Restorative Action of Mn^{2+} on Membrane Potential of Rat Liver Mitochondria De-energized by Ca^{2+} and Phosphate Cycling

A. TONINELLO, F. DI LISA and N. SILIPRANDI

Centro Studio Fisiologia Mitochondriale C.N.R. Padova e Istituto Chimica Biologica, Università di Padova, Padua, Italy

Inorganic phosphate and Ca^{2+} in the incubation medium induce a parallel efflux of endogenous Mg^{2+} and adenine nucleotides from rat liver mitochondria [1]. At first no release of accumulated Ca^{2+} occurs during Mg^{2+} efflux; however as soon as 50% of endogenous Mg^{2+} has been lost, Ca^{2+} begins to escape as well. It was also shown that addition of Mg^{2+} to deenergized mitochondria restores the original membrane potential and confers to mitochondria the full capacity to reaccumulate the Ca^{2+} lost [2]. Here, we report that the membrane potential of liver mitochondria incubated in the presence of Ca^{2+} and phosphate is preserved by Mn^{2+} and restored by the same cation plus ATP or ADP when collapsed. In the typical experiment reported in Fig. 1 $\Delta\psi$ of

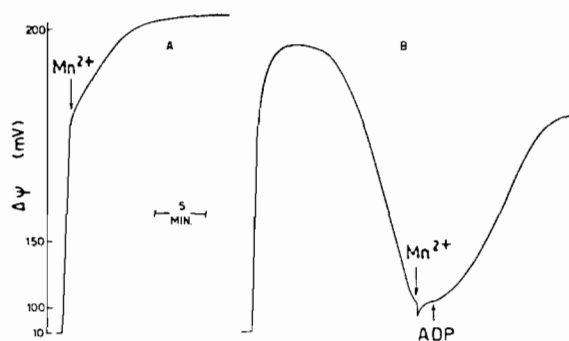


Fig. 1. A) Prevention by Mn^{2+} of $\Delta\psi$ collapse induced by Ca^{2+} and phosphate cycling. B) Restoration by Mn^{2+} of $\Delta\psi$ collapsed by the action of Ca and phosphate.

liver mitochondria, incubated in the presence of Ca^{2+} and Pi was fully preserved by $50 \mu M Mn^{2+}$. Furthermore, when Mn^{2+} was added to mitochondria deenergized by the action of external Ca^{2+} and phosphate, Mn^{2+} restored the original $\Delta\psi$ provided that ADP was also added. Concordantly, as shown in Fig. 2, Mn^{2+} prevented the parallel efflux of endogenous Mg^{2+} and adenine nucleotides induced by the flux of Ca^{2+} and phosphate. This action of Mn^{2+} is very similar to that of Mg^{2+} with three major differences: (1) Mn^{2+} is active at much lower concentrations; Mg^{2+} exhibits the same action [2] of $50 \mu M Mn^{2+}$ when added in concentrations above 1 mM. (2) Unlike Mg^{2+} , Mn^{2+} restores collapsed $\Delta\psi$ only when added together with ATP or ADP. (3) Mg^{2+} are unable to restore collapsed $\Delta\psi$ if Mn^{2+} are previously added.

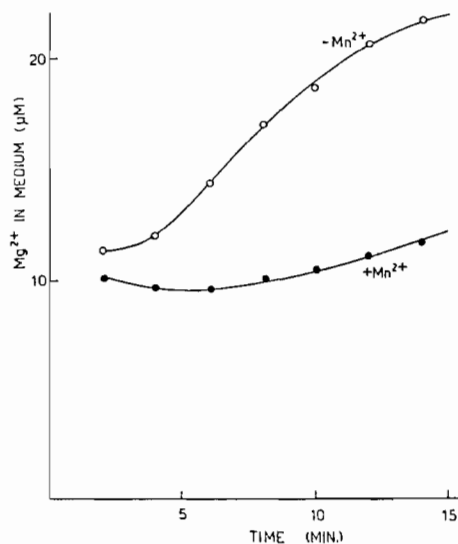


Fig. 2. Inhibition by Mn^{2+} of Mg^{2+} efflux induced by Ca^{2+} cycling.

These results show that Mg^{2+} can be replaced by Mn^{2+} in some of their roles in mitochondria and

provide preliminary evidence that Mg^{2+} and Mn^{2+} compete for the same binding sites located on the inner mitochondrial membrane. This assumption explains the inability of Mg^{2+} to act once Mn^{2+} has been added to the mitochondrial suspension.

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S8

Iron Transport and Accumulation in Mammalian Cells

ROBERT R. CRICHTON

Unité de Biochimie, Université Catholique de Louvain, Belgium

Iron is essential for all cells and mammalian cells are no exception. The daily requirements for iron are provided to a small extent from absorption of dietary iron essentially in the upper part of the intestine, to a large extent mediated by mucosal transferrin, and by reutilisation of tissue iron, to a major degree furnished by the breakdown of effete red blood cells, and assured by the serum β -globulin transferrin. In fact of the 35 mg or so of iron which exchanges between different body compartments each day, only 1 mg is contributed by mucosal iron absorption, and this latter is compensated by an equivalent amount of iron excretion (also about 1 mg/day). We shall concern ourselves here with three aspects of iron metabolism in mammalian cells: (i) iron uptake by cells from serum transferrin (ii) accumulation of iron in the intracellular storage protein ferritin (iii) intracellular transport of iron to sites where it is required for essential cellular functions such as haem synthesis.

Iron uptake from serum transferrin. It has been well established for many years that many mammalian cells (indeed probably all cells) have plasma membrane receptors for transferrin, and the transferrin receptor has recently been purified and characterised [1]. It is an integral membrane protein of MW 180.000 composed of two disulphide linked subunits which are each capable of binding one transferrin molecule (MW 80.000 bearing one or two iron atoms). Subsequent to binding to its receptor, the transferrin-receptor complex is internalised by endocytosis and its iron is released by protonation of the bicarbonate (or carbonate) anion bound to the iron atom [2]. The apotransferrin molecule is recycled to the plasma membrane and released for reutilisation. In some cell types an additional mechanism intervenes in which the transferrin molecule is digested in lysosomes subsequent to iron release.

Accumulation of iron in ferritin. The intracellular iron storage protein is ferritin which provides a bio-available, soluble and non-toxic form of iron essentially as hydrolysed ferric oxyhydroxide enclosed within a globular protein shell (apoferritin) of MW 480.000. The amino acid sequences of human and horse apoferritins have been determined [3, 4] and the X-ray structure of horse spleen protein is well advanced [5]. The mechanism of iron deposition involves catalysis by the protein of the oxidation of Fe II bound to adjacent subunits, which may involve peroxo-bridged and μ -oxo intermediates prior to hydrolysis. Subsequent events may occur at the polymer surface or be catalysed by the protein and our current understanding of the incorporation of iron in ferritin will be reviewed.

Intracellular Iron Transport for Haem Synthesis. The ferrochelatase of the mitochondrial matrix catalyses the final step in haem biosynthesis, namely the incorporation of Fe II into protoporphyrin IX. Recent studies suggest [6, 7] that ferritin may well be the source of ferrous iron for haem synthesis via reduction of ferritin iron by reduced flavins generated by electrons from the respiratory chain, and implying a rather specific interaction of ferritin with the mitochondria. Recent results in this area will be presented and the role of ferritin in intracellular iron transport will be reviewed.

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S9

Vanadium Absorption by Plants: the Uptake of Vanadium by Excised Barley Roots (*Hordeum Vulgare* c.v. *Maris Mink*)

N. W. LEPP, B. G. MORRELL

Department of Biology, Liverpool Polytechnic, Byrom Street, Liverpool, L3 3AF, U.K.

and D. A. PHIPPS*

Department of Chemistry and Biochemistry, Liverpool Polytechnic, Byrom Street, Liverpool, L3 3AF, U.K.

The absorption of vanadium by plants has received only limited attention [1]. However it presents an