contents of Thr, Ser, Ala and Cys residues were remarkably different.

1 H. Kito, Y. Ose, V. Mizuhira, T. Sato, T. Ishikawa and T. Tazawa, *Comp. Biochem. Physiol., 73C*, 121 (1982).
2 Y. Kojima, C. Berger, B. L. Vallee and J. H. R. Kagi, *Proc. Natl. Acad. Sci., 73*, 3413 (1976).

S5

Mobilization of Cu(II) from Plasma Components and the Mechanism of Cu(II) Transport by Rat Hepatocytes

H. M. DARWISH, J. C. CHENEY, R. C. SCHMITT and M. J. ETTINGER

State University of New York, Buffalo, N.Y. 14214, U.S.A.

Albumin and amino acid bound Cu are the readily accessible forms of plasma Cu with a half-life of ~10 min [1]. Hepatic uptake largely accounts for this rapid clearance of exchangeable plasma copper [2]. We have recently characterized the kinetics of a Cu-specific transport protein of rat hepatocytes [3] and here report the effects of plasma components.

Albumin markedly inhibits ^{64}Cu(II) uptake at up to 10:1 molar excesses of Cu. In the presence of albumin, the nonlinear Lineweaver-Burk plots obtained converge to the same V_{max} and K_m parameters as for free Cu which indicates inhibition by a substrate-removal mechanism. Histidine facilitates albumin-inhibited Cu(II) uptake, but rates of Cu uptake in the presence of histidine do not exceed the rates for free Cu(II). Several (10) amino acids were tested including Thr and Gln which have been detected in Cu-complexes isolated from plasma [4], but only histidine facilitated albumin-inhibited Cu-uptake. Moreover, the facilitating activity of a low molecular weight ($\leqslant$5000 daltons) rat plasma fraction was accounted for by its histidine content. The tripeptide, Gly-His-Lys which was reported to facilitate Cu uptake in hepatoma cell cultures [5] had inhibitory activity similar to albumin.

Albumin was dialyzed with ^{64}Cu plus [^{3}H]-His, and the transport activities of the albumin-containing and albumin-free fractions in equilibrium were compared. Transport activity was completely accounted for within the excess histidine plus ^{64}Cu(II) fraction. At pH 7.4, the predominant species was $His_2Cu(II)$ [6]. This complex exhibited the identical V_{max}, but higher (20 *vs.* 10 μM) K_m as free Cu(II). Given the stability constant of $His_2Cu(II)$ ($\beta_{102} \simeq 10^{18}$) [6], the transport activity of the complex cannot be accounted for by free Cu(II) in equilibrium with the complex. Copper uptake experiments with [^{3}H]-His_2^{64}Cu(II) showed that Cu and His are not co-transported. Thus, histidine apparently facilitates Cu uptake by competing with albumin for Cu. The results are consistent with binding of the $His_2Cu(II)$ complex to the Cu-transport protein, a ligand-exchange reaction, and transport of free ionic Cu.

1 N. Marceau, N. Aspin and A. Sass-Kortsak, *Amer. J. Physiol., 218*, 377–383 (1970).
2 A. Sass-Kortsak, *Adv. Clin. Chem., 8*, 1–67 (1965).
3 R. C. Schmitt, H. M. Darwish, J. C. Cheney and M. J. Ettinger, *Amer. J. Physiol.*, in press (1983).
4 B. Sarkar and T. P. A. Kruck, in 'The Biochemistry of Copper', J. Peisach, P. Aisen, W. E. Blumberg (eds.), Academic Press, New York and London, pp. 183–196 (1966).
5 L. Pickart, J. H. Freedman, W. J. Loker, J. Peisach, C. M. Perkins, R. E. Stenkamp and B. Weinstein, *Nature, 288*, 715–717 (1980).
6 R. J. Sundberg andR. B. Martin, *Chem. Rev., 74*, 471–517 (1974).

S6

Bioinorganic View of Evolution: the Case of Copper

EI-ICHIRO OCHIAI

Department of Chemistry, Juniata College, Huntingdon, Pa. 16652, U.S.A.

Since organisms depend on a number of elements in addition to those contained in organic compounds, the biological evolution may be studied from the viewpoint of the interaction between inorganic elements and the biological systems, *i.e.*, bioinorganic chemistry.

The bases of this approach [1] are (1) differential requirements for elements (different organisms may need different elements), and (2) the historical variation in the availability of elements on the earth, especially in the hydrosphere. One fundamental assumption is that an organism which would require a specific element would not evolve (come into being) before that element becomes readily available to it. The historical variation in the availability of elements depends mainly on the oxidative state of the hydrosphere, which in turn may be controlled by the oxygen content of the atmosphere. The latter is believed to have changed substantially during the course of earth's history, from a very low value at the beginning to the rather high value in the present atmosphere. Accordingly the oxidation states of elements could have been altered throughout.

These principles are illustrated here by the case of copper. Copper, because of its rather high reduction potential (E for Cu(II)/Cu(I) = +0.34 v at pH = 0), seems to have been unavailable (in the soluble Cu(II) form) until quite late in the history of the earth. An estimate [1, 2] puts the time when Cu(II) became readily available in the hydrosphere

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.

1 E.-I. Ochiai, *Origins of Life, 10*, 81–91 (1978).
2 E.-I. Ochiai, in 'Cosmochemistry and Origin of Life', C. Ponnamperuma (ed.), in press (1982).

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 Mitocondriale 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

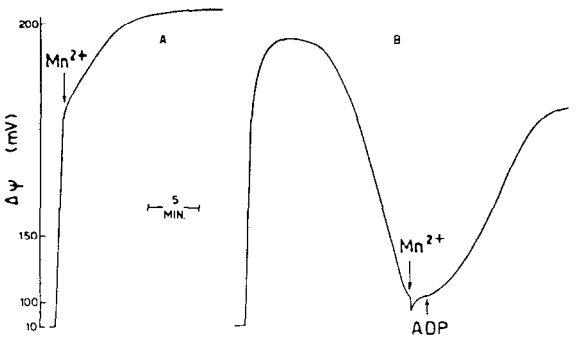

Fig. 1. A) Prevention by Mn^{2+} of $\Delta\psi$ collapse induced by Ca^{2+} and phosphate cycling. B) Restoration by Mn^{2+} of $\Delta\psi$ collapsed by the action of Ca and phosphate.

liver mitochondria, incubated in the presence of Ca^{2+} and Pi was fully preserved by 50 μM Mn^{2+}. Furthermore, when Mn^{2+} was added to mitochondria de-energized 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 μM 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.

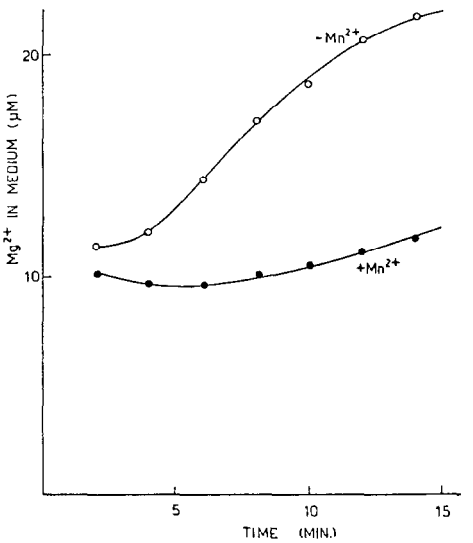

Fig. 2. Inhibition by Mn^{2+} of Mg^{2+} efflux induced by Ca^{2+} cycling.

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