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

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S5

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

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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.

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S6

Bioinorganic View of Evolution: the Case of Copper

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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