presence of rose bengal. In this case the formation of new hydroperoxides occurred also when the photoactivation was ended because of the branching reactions initiated by decomposition of the lipid hydroperoxides previously formed by rose bengal. The chelating agent EDTA lowered the rate of lipid hydroperoxide formation due to branching reactions, but did not decrease the amount of preformed lipid hydroperoxides. On the contrary, Mn^{2+}, like the antioxidant agent butylated hydroxyanisole (BHA), decreased them.

On this ground we suggest that Mn^{2+} could directly interact with the oxy and peroxy radicals ($RO^{\bullet}$, $RO_2^{\bullet}$) originating from lipid hydroperoxide decomposition. Consequently these free radicals cannot spark new radical chains, and lipoperoxidation is blocked.

Recently Mn^{2+} was shown to act as a scavenger of $O_2^{\bullet-}$ [4] and $^{\bullet}OH$ radical [5] and this supports the hypothesis of a direct antioxidant action of this ion.

The inhibitory action of Mn-superoxide dismutase (SOD) on lipoperoxidation was also studied. The enzyme inhibits by 25–30% the lipid peroxidation sparked by CHP in experiments carried out in the presence of reduced glutathione.

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S4

Amino Acid Compositions of Carp Kidney Metallothionein

HIDEAKI KITO, YOUKI OSE, TAKAHIKO SATO, TETSUYA ISHIKAWA, MASAKO KINOSHITA and KAORI KONDO

Gifu College of Pharmacy, 6-1, Mitahora-higashi 5 chome, Gifu, Japan

SYOJI YONEZAWA

Nagoya City Health Research Institute, 1-11, Hagiyama-cho, Mizuho-ku, Nagoya, Japan

In fish, homology on amino acid compositions of metallothionein (MT) between liver and kidney is not recognized. We previously reported the amino acid composition of carp hepato-pancreas MT [1]: in the present study amino acid composition of kidney MT was determined, and homology between them discussed. Twenty carps were injected intraperitoneally with 2 mg/kg body weight of Cd as $CdCl_2$ solution, daily for 3 days. On the fourth day the kidney was excised, treated successively by homogenization, ultracentrifugation, heating treatment, centrifugation, gel filtration (Sephadex G-75, Bio Gel P-10) and ion exchange chromatography (DEAE Sephadex A-25). By the gel filtration using Sephadex G-75, the Cd peak was observed, and it was estimated that this peak contained MT. By ion exchange chromatography this peak was separated into two fractions, having high concentration of Cd, and high absorption at 254 nm and no absorption at 280 nm respectively. The former fraction was named MT-I and the latter MT-II. By gel filtration using Bio-Gel P-10, a single Cd peak was observed in MT-I and MT-II, respectively. The molar ratio of Cd: Zn was 36:1 in MT-I and 27:1 in MT-II. Their purity was shown by polyacrylamide DISC-gel electrophoresis. The absorption at 254 nm in both MT fractions disappeared when they were brought to acidic condition. The amino acid compositions of carp kidney and hepato-pancreas are given in Table I. Cysteic content was about 30% in each organ. The contents of Lys, Glu, Pro and Met residues in carp MT were close to the values in mammal MT, but those of Thr and Ala residues were different. In kidney MT, some Leu residue was observed. Arginine residue was detected only in kidney MT-II. No aromatic amino acids and histidine residues were detected in carp MT. The amino acid compositions in kidney MT were little different from those of hepato-pancreas MT. Between MT-I and MT-II in kidney the

TABLE I. Amino Acid Compositions of Carp Kidney and Hepato-pancreas.

Amino acid	Mol % (number)				
	Carp				Horse [2]
	Hepato-pancreas		Kidney		Kidney
	MT-I	MT-II	MT-I	MT-II	
Lys	11.37(7)	10.09(6)	10.06(6)	10.09(6)	11.1(7)
His	– (0)	– (0)	– (0)	– (0)	– (0)
Arg	– (0)	– (0)	0.51(0)	0.81(1)	1.9(1)
Asp	8.47(5)	9.10(6)	8.04(5)	7.53(4)	5.0(3)
Thr	7.10(4)	8.47(5)	6.68(4)	9.48(6)	1.8(1)
Ser	11.23(7)	10.13(6)	6.99(4)	11.78(7)	11.9(8)
Glu	3.11(2)	3.50(2)	5.40(3)	4.29(3)	4.7(1)
Pro	4.89(3)	4.16(3)	3.55(2)	3.85(2)	2.9(2)
Gly	10.64(6)	11.13(7)	9.30(6)	11.51(7)	9.2(5)
Ala	5.82(3)	5.59(3)	8.43(5)	6.05(3)	11.6(7)
Cys	34.24(20)	31.90(20)	32.70(21)	29.39(18)	33.9(20)
Val	1.98(1)	2.35(1)	2.95(2)	2.98(2)	4.5(3)
Met	1.01(1)	1.68(1)	0.96(1)	0.50(1)	1.5(1)
Ile	0.11(0)	0.99(1)	1.67(1)	0.70(1)	– (0)
Leu	– (0)	– (0)	2.74(2)	1.14(1)	– (0)
Tyr	– (0)	– (0)	– (0)	– (0)	– (0)
Phe	– (0)	– (0)	– (0)	– (0)	– (0)

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

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.

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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