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The Role of Divalent Metal Ions in the Mechanism of Action of the Hormone Angiotensin-II

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The pressor and myotropic activities *in vivo* of the linear octapeptide hormone Angiotensin-II (A-II) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) are enhanced by divalent metal ions. In model membrane systems consisting of phospholipid vesicles, A-II behaves as ionophore in translocating Mn(II) ions across the bilayer [1] with a rate constant which is pH dependent with an apparent pK of 7.7. Since A-II is itself interacting with the bilayer in a pH dependent way [2], the metal–A-II coordination modes are expected to play a central role in the biological effect of the hormone.

In this report nuclear and electron relaxation features of the A-II–Mn(II) complexes in aqueous solution are measured with the aim of delineating the metal binding sites.

The ESR intensity loss and the appearance of 'slow motion' features in the X-band ESR spectrum of Mn(II) were taken to demonstrate a main binding interaction with one (or two) carboxyl groups, since the ESR parameters were pH independent over the whole range investigated (pH = 4–8).

The paramagnetic contributions to nuclear relaxation rates, T_{1p}^{-1}, were measured for aromatic protons of A-II (a typical spectrum is shown in Fig. 1) and, since $T_{2p}^{-1} < T_{1p}^{-1}$ for all the protons, i) fast exchange conditions were assumed and ii) dipolar only contributions to T_{1p}^{-1} were considered. The T_{1p}^{-1} and T_{2p}^{-1} values were therefore taken to demonstrate that the imidazole moiety of His_6 is a secondary metal binding site.

The pH dependence of T_{1p}^{-1}, reported in Table I, clearly showed that the binding to His_6 is relevant in a narrow pH range only, which could be explained in

TABLE I. pH dependence of T_{1p}^{-1} for Phe and His H_2 protons of A-II. [A-II] = 0.01 *M*; [Mn^{++}] = 0.01 m*M*; T = 295 K.

pH	T_{1p}^{-1} (sec^{-1})	
	Phe	His H_2
4.5	0.12	0.14
5.5	0.18	0.62
6.0	0.22	1.83
6.5	0.24	6.22
7.0	0.22	4.90
7.5	0.19	2.05
8.0	0.14	1.62

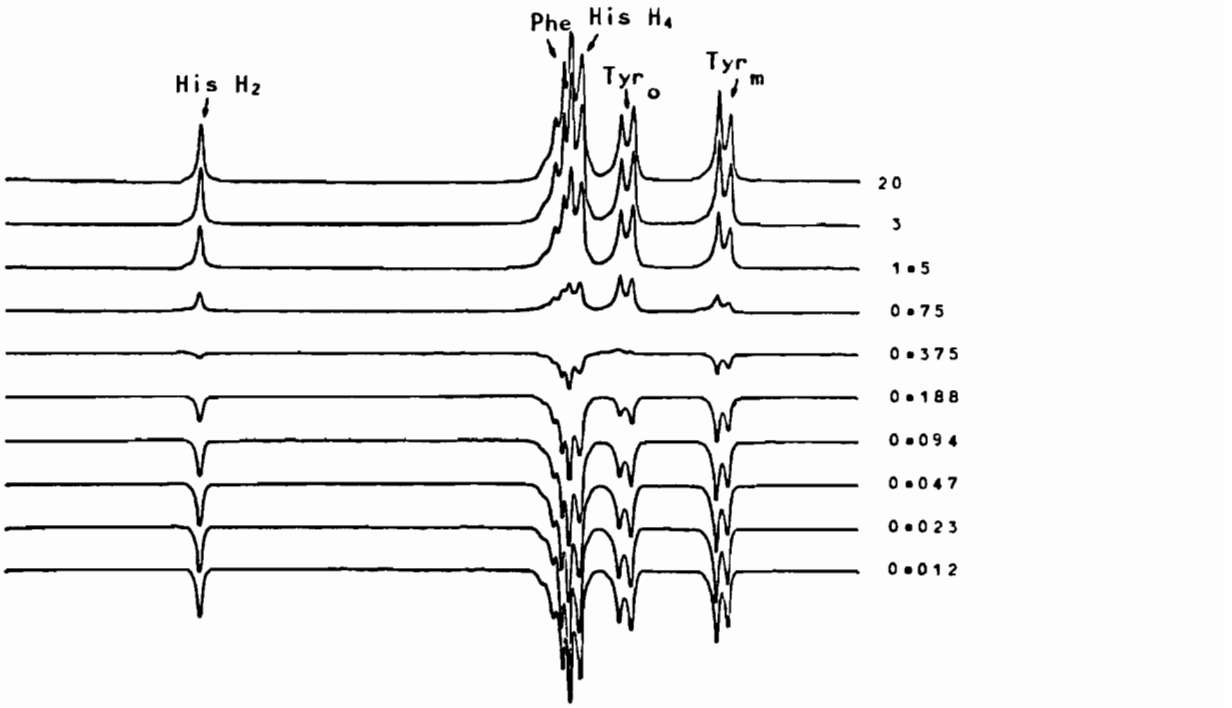

Fig. 1. PRFT proton NMR spectra of the low field aromatic region of A-II 10 mg/ml in D_2O. T = 295 °K. pH = 7.0.

terms of intramolecular structuring within the peptide molecule opposing metal coordination driven by deprotonation of the imidazole ring.

1 H. Degani and R. E. Lenkinski, *Biochemistry, 19,* 3430 (1980).
2 G. Valensin and H. Degani, *FEBS Letters,* submitted.

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Possible Applications of Coordination Compounds for Correcting Biometal Metabolism in Different Pathologies

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During the last two decades laboratory (with animals) and clinical researches have shown that many pathologic states of a body are accompanied by statistically reliable disturbances in the metabolism of metals at the molecular and body levels. Any chronic disease, the cause of which has not yet been established, can be due to abnormalities in metal metabolism. The determination of the amount of biometals in the body is suggested as the earliest diagnostic test of diseases.

The metals participating in metabolism can be divided into the following groups:

(1) inherent in a living body and involved in the sphere of essential biofunctions (Cu, Fe, Zn, Mn, Mo, Co, Mg, Ca, K, Na);

(2) introduced, often toxic, whose physiological role has not been fully elucidated and their presence in the body tissue and liquids is due to their abundance in nature and wide application by people (Al, Cr, Cd, Ni, Pb, *etc.*).

For the 1st group of metals both positive and negative balances were detected in different pathologies, and for the 2nd group, as a rule, only the positive balance was observed.

One of the reasons for the abnormal accumulation and removal of metals from a human body may be the wide application of drugs in clinics and which, by their chemical nature, are good ligand–complexing agents (up to 80% of all used drugs). Using non-steroidal antiinflammatory compounds (HL) and a copper-containing blood enzyme, ceruloplasmin (CuCPL), the ligands (drugs) were shown to take away competitively the metals from metal-containing and metal-activating enzymes:

$$CuCPL + HL = CuL + CPL$$

Such an interaction results in a 'discomfort' of an enzyme system in the body which is indicative of a side effect of drugs, *i.e.* complexing agents.

For some diseases the shifts in metal metabolism are specific: rheumatoid arthritis—(–) Fe, Zn; (+) Cu, Al, Mn, Mo, Cr; atherosclerosis—(–) Cr, Mn, Zn; cancerogenesis—(–) Cu, Fe, Mg; (+) Zn, Mn; diabetis—(–) Cu, Mn, Cr; (+) Zn; *etc.* The correction in the concentration of these metals results in a therapeutic effect.

The complex compounds of biometals with different types of drugs are the most promising tool for introducing the required metal into the body. It has been established that the application of antiinflammatory agents as complexes with some biometals decreases their toxicity and increases and prolongs their therapeutic effect (chemico-therapeutic synergism); antiulcerogenic, cytotoxic and other helpful properties, unusual to non-complexed agents, appear.

It has been shown that a probable form of storage and transport of cardiovascular biogenic amines is their highly stable complexes with essential biometals and ATP.

In all cases the ligands (drugs) in the composition of metallocomplexes cause no decomposition of endogenic metalloferments and have no side effects.

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Therapeutically Active Cu(II), Zn(II) and Fe(III) Complexes with N-phenylanthranilic Acid Derivatives and Their Effect on Redox Reactions Modeling Inflammation

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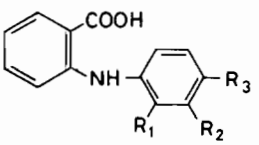
The amount of copper(II), zinc(II) and iron(III) involved in the majority of metalloferments during different diseases of inflammatory nature varies more than that of other metals.

The possibility of bonding the metal involved in ferments by drugs of acidic nature has been established by studying the interaction between ceruloplasmin (CP) and N-phenylanthranilic acid derivatives (HL), antiinflammatory agents. The reaction



HL