METAL IONS AS A FACTOR OF FUNCTIONAL EVOLUTION AND OF DEVELOPMENT OF SOME IMPORTANT BIOCHEMICAL PROPERTIES IN PREBIOTIC AND BIOLOGICAL CONDITIONS.

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Arguments are given why metal ions could be considered as a prototype or factor for the emergence of biologically important functional systems. The transmission of their properties to the considerably more complex metal-proteins and the prospect for new functional possibilities to emerge in the process of metal ions-protein linking are considered to be essential. A series of mechanisms by which metal ions participate in the specialization and modification of the catalytic function, in the perfection of metabolic control,are displayed.

The appearance of life on Earth may be considered from a functional point of view as emergence and integration of important functions in a common manifestation of the vital activity of the biological system. Precisely it is the bearer of the most essential differences between living and non-living matter. Nevertheless, this aspect of the biogenesis and evolution of life has been relatively less elucidated and no specific regularities have been established. Considerably more is known about the structural and morphological changes,about the substantial changes upon the trasition from non-living to living matter in the course of evolution.

The first and possibly the most essential thing which should be pointed out in connection with the development of the functions is that none of them could emerge all of a sudden as a completely new phenomenon. Consequently, the search for continuity and respectively for a prototype of a given biological function or of a given biological pathway is in general a necessary approach in studying functional evolution.

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Moreover, it is particularly important also to seek those new moments which appear with respect to a given function or are reflected on some more integral function, changing it to a certain extent, e.g. the ability for adaptation. Another aspect deserving attention consists in the causes leading to increased complexity and intensified biological activity of each function separately, as well as jointly, of the functional activity of the living system. In fact, the question arises here about the hierarchy of functions really existing in each living system. The foundation of this hierarchy should be the functions inherent to the basic cell macromolecules, followed by the functions of the various organelles, tissues, plant organs and finally the global vital functions of the organism. Clearly this is a case of a functional integration, closely related and in its turn conditioning an effective regulatory system. There are grounds to agree with Koktaiskiy (1) who stresses that the hierarchy of functions corresponds to the hierarchy of the structural levels. This assertion corresponds to the logic of structural-functional unity.

However, as it has already been mentioned, each function, irrespective of the level of its manifestation, undergoes changes and perfectioning in the course of evolution. Precisely these processes and tendencies are particularly important for the understanding and elucidation of the principles of functional evolution in general. Probably they are most clearly manifested at the levels of macromolecules and organisms. Special attention should be devoted to those factors which are related to the manifestation, modification, specialization and the so-called "expansion" of the function. From this point of view our studies for many years on the biological role of the metal ions in the plant organism gave us grounds for assuming that they are a very important factor of this type.

In order to throw light on this question we performed a detailed survey of the data in the available literature about the role of the metal ions in the primary synthetic reactions, in the primitive syntheses of organic substances in the prebiological stage, as well as during the initial integration of the macromolecules (2,3). It proved possible to deduce the main functional characteristics of the metal ions in the primary broth, which were of great importance for the emergence of life. This made it possible to compare the observed functions and the functions of the metal ions in the present-day plant organism (Table 1). Several aspects of the comparison deserve special attention. Above all, one should note the undisputable uniformity of the functions and in some cases the indisputable analogy of most of them. This leads to several more important conclusions:

1. A large number of the metal ions are a functional prototype of a number of functions typical of the biological mac-

romolecules;

2. To a great extent upon the transition from non-living to living matter precisely the metal ions attribute their primary and naturally more primitive functions to the more complex biochemical systems, thus achieving the continuity in the development of the functions;

3. During the later stages of the evolution, when the functional units are considerably more complex and comsist of biological macromolecules and macromolecular complexes, the metal ions play a role in the development, modification, specialization, scope and integration of the functions.

TABLE 1
Main Functions of Metal Ions (3)

IN THE BIOPOIESIS:	IN THE PRESENT-DAY ORGANISMS:
1. Catalytic, non-enzymic catalysis	1. Catalyitic: a/ non-enzymic catalysis b/ enzymic catalysis
2. Electron-transport	2. Electron-transport
3. Structural, determine the conformation of organic compounds and primary polymers	3. Structural, determine the conformation of the macromolecules
4. Create structural and functional variety, favour natural selection at molecular level	4. Create structural and functional multiplicity of macromolecules, favour molecular selection and adaptation processes
5. Determine the way of primary syntheses	5. Determine the direction of metabolic pathways
6. Photosensitisation in primary reactions	6. Photosensitisation effect on many organic compounds
7. Integrative function in the self-assembly of macromolecules	7. Integrative function in the self-assembly of cell organells and organisation of metabolism
8. Factors for the emergence of molecular asymmetry	8. Factors of the molecular asymmetry in the living sytems
9. They played the role of inorganic matrix	9. Participate in the creation of organic matrix
	10. Initiate additional interactions with the macromolecules, increase the effectiveness of metabolism and the adaptive capacity of the system.

These three conclusions show that metal ions can indeed be considered as a factor and an important prerequisite for the appearance and evolution of the main biological functions.

The evidence which served for the formation of Table 1 as regards the prebiotic functions is considerable and convincing. We took into account the data about the metal-dependant synthesis of aminoacids (4,5), about the participation of metals in the primary formation of polyphosphates (6,7,8), in the synthesis of polypeptides (9,10,11 and others). A very large number of metal ions are indicated as participants in the abiotic synthesis of porphyrins (12,13 and others).

Data in the literature on the role of metals in prebiological systems are very scanty. Nevertheless, it is possible to find indications that the metals may undertake some function which affects the general stability of the system or provoke the apperance and development of an important biological function. Thus, for example, Evreinova et al. (14) have found that the stability of the coacervate depends on the inclusion of some enzyme, e.g. peroxidase, in it. It is not unreasonable to speculate that the presence of a metal with the same catalytic properties might lead to the same result. Of importance for the stability of the coacervate drop is also the correlation between the magnesium ions and the various nucleotides (15). Zinc ions facilitate the apperance of phosphatase-like activity in the proteinoid microspheres- whereas the inclusion of calcium ions stimulates division (16,17). Consequently, here it is possible to see the initial moment in the participation of metal ions in the emergence of the function, regulation and integral behaviour of the system. In other words, the integrating function of metal ions begins to be manifested even in the prebiological systems.

In the typical biological evolution the metal ions influence the functional evolution at different levels, though particularly important is their role in regulation and evolution at molecular level. It is performed by interaction with the main biological macromolecules, particularly protein. Naturally, however, the effect obtained at this level is reflected in higher levels and this is probably one of the mechanisms through which metal ions influence the general functional activity of the system as a whole. For example, in many cases the changes in the isoenzyme spectrum of peroxidase are connected with the adaptive processes in the plant organism.

As we have pointed out on many occasions (2,3,18) in the evolution of the biological catalysis metal ions are related both with the determination of the basic functional characteristics of metalprotein and with the increase in the effectiveness of the biological catalysts. This effect is based on their interaction with protein. In the evolution of catalytic proteins there is no essential change in the main function of the metalproteins with catalytic or electron-transport functions, formed during the early stages of biogenesis. Along general lines, they are determined by few but strictly defined elements: copper, iron, cobalt, molybdenum, nickel and manganese. However, at the same time additional, secondary and even tertiary changes in the molecule, caused by the same or other metals, may take place, and these changes play an exceptionally important role in the development, perfectioning and realization of the functions, as well as in the realization of the basic principles of functional evolution.

If the primary function of the metalprotein originates from the prebiological properties of the metal ions (and it is known that a number of metals have enzyme-like functions), then the additional interactions with other types and with a greater number of metal ions introduce something new in the manifestation of the function, even the appearance of additional properites of the macromolecules. All this enriches the possibilities for choice of the course of the metabolic processes, and it is reflected in the integral metabolism of the cell, which was of great significance in the evolution of the species. On the basis of these considerations it may be concluded that since the earliest stages of the biological evolution the metal ions are established as a component of the regulatory system of the plant cell. Their most characteristic feature is their ability to create fine regulatory mechanisms. Thus, metals become obligatory biologically necessary components of each living system. In this way it is possible to explain the specificity in the elementary composition of living systems, the concentration of certain elements in definite cell organelles, the necessity of a definite element for the realization of a definite metabolic pathway, the changes in the requirements of the living system to one element or another in the course of evolution.

Owing to the secondary or additional interactions between the metal ions and the protein, which result in inessential structural but important functional changes, there appear also a number of new aspects and possibilities in the functional characteristics of the protein molecule. In fact, they determine to a great extent the evolution of the function at molecular level. From our studies over many years and on the basis of the data in the literature, it may be concluded that metal ions are related to the following aspects of the functional evolution:

1. Appearance of additional functional activity;
2. Adaptation of the function to the metabolic specificities of a given species;

3. Specialization of the functions with the advantages and disadvantages ensuing from it;

4. Changes and evolution of the enzyme heterogeneity;

5. Modification of the function - an extremely important characteristic, related to the adaptation and perfectioning of the biological systems;

6. Perfectioning of the mechanisms of control at molecular and cellular levels;

7. Control of the polyfunctionality;

8. Expansion of the function.

In fact, all these features are very closely related, which is of great biological significance, because in this way it is possible to guarantee the simultaneous realization of two very important aspects of the evolutionary process: specialization related to the perfectioning of the function and the ability for adaptation, which requires the existence of more than one possibilities or a certain variability of the system. As Baianu (19) has pointed out, the functional approach in biology requires to take into account the partial disorder in a structural and partially in a functional respect.

Concrete evidences related to the abovementioned features were obtained in studying plant peroxidase - a typical metalprotein. The basic function of this enzyme system is to catalyze the oxidation of various substrates in the presence of hydrogen peroxide or other peroxides. However, it has been proved that the peroxidase catalyzes other oxidative reactions as well, but already in an oxidase way, i.e. by using molecular oxygen. One of the earliest studied reactions is the peroxidase-catalyzed oxidation of indolylacetic acid. In addition, plant peroxidase possesses also ascorbate oxidase properties (20). Consequently, peroxidase is a very suitable model system, possessing polyfunctionality, considerable heterogeneity and adaptive properties. In studying the effect of metal ions on the catalytic properties of this enzyme system, we obtained evodence about the fact that the entire functional activity of peroxidase is conditioned by metal ions. On the other hand, the iron in the active centre is resposible for the peroxidase and oxidase properties, but the degree of realization of the function depends also on the presence of other types of ions as well. This is valid both in the ontogenesis and in the manifestation of the function at a given stage of phylogenesis, in the appearance of new metabolic situations or principles.

The established role of the metal ions in the cell as metabolic effectors of a number of enzyme systems contributes to the adaptation of the catalytic function to the metabolic peculiarities of the species, on the one hand, and on the other - it controls the correlation of the functions (through inhibition of one function and activation of another), and consequently the polyfunctionality - thus being directly related to the principle of "expansion" or "narrowing" of the function in the course of evolution. The principle of "expansion" of the function is interpreted mainly at the level of organ changes in the phylogenesis of animals, presuming that it is based on an increase in the multifunctionality and on the intensification of the functions under unstable conditions (22). Our studies have shown that the metal ions are a factor in the realization of this priciple at the level of catalytic proteins and that they control the behaviour of the polyfunctional system by increasing or in isolated cases by limiting the functional possibilities.

The control on the polyfunctionality of the enzyme through metal ions is achieved in two ways:

1. <u>Influence in different directions on the various types of activity</u>. Under the conditions of our experiments calcium ions are typical activators of the IAA-oxidase function of peroxidase, whereas zinc and copper ions - control the ascorbate oxidase function.

2. <u>Incorporation of new portions of protein in a given catalytic reaction</u> - this is achieved by activation of predominantly weakly active or inactive protein portions by a given ion which provokes the acquiring or manifestation of one catalytic function or another.

A very important aspect of the biological role of metal ions is their ability to produce structural and functional multiple forms at different levels, above all at molecular level. It is possible to observe changes in the isoenzyme composition of many enzymes, peroxidase included. Metal ions controll the differences in the functional characteristics and in the correlation of the functions of the individual isoenzyme components. This is a mechanism controlling the scope of the entire function. Another mechanism,related to the principle of expansion of functions, is the possibility of simultaneous parallel action of different types of catalyzers with equal functional characteristis (e.g. metalproteins, metalorganic complex compounds and metal ions). We obtained indications that such a situation could exist in the plant cell (3,18).

In some cases different enzyme systems can catalyze one and the same reaction. The reduction of nitrates in plants takes place with the participation of the enzyme nitratereductase which is connected with molybdenum. The nitrate-reducing function is also characteristic of peroxidase which is an iron-containing protein. It is difficult to determine the normal share of peroxidase in nitrate reduction, though it should not be underestimated as a reserve possibility. This is probably the case of another principle - guaranteeing the function.

The facts and considerations presented here give us grounds to assume that metal ions are an essential factor of the evolution of functions in living systems. Since the earliest stages of the biogenesis they are related to the emergence and development of functions of great biological importance. That is why metal ions were indispensable for the transition from prebiological to biological systems. They still continue to play an essential role in the structural and functional evolution of life at different levels of organization.

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