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Metal ions as enzyme effectors

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Abstract. The role of metal ions as enzyme effectors is considered. Data on inhibitory and activating effects of metal ions are summarised. The dual character of action of the effectors depend ing on their concentration and the nature of the enzyme is highlighted. The analytical applications of these effects are discussed. The bibliography includes 66 references.

I. Introduction

The role of metal ions in the mechanism of catalytic effects of enzymes is diverse.[1] One group of enzymes is characterised by the presence of one or several tightly bound metal ions involved in catalytic processes (metalloenzymes). Other enzymes do not contain directly bound metal ions but manifest catalytic activity only when the ions are present (metal-dependent or metal-activated enzymes). For enzymes of the third group, metals are not obligatory participants of the catalytic reaction; however, their presence in the system influences the enzyme activity (metal-independent enzymes).

Three types of metal – enzyme complexes are generally formed in enzymic catalysis:

(1) Complexes through the ligand L (substrate) as a bridge. Here, the metal ion binds only to the ligand and takes part in the catalysis not being directly bound to the enzyme (Fig. 1, pathway *I*). These complexes are typical of many enzymes, *e.g.*, creatine kinase,[2,3] and some other kinases and synthetases.[4–6]

(2) Complexes in which the metal ion binds the ligand completely (Fig. 1, pathway *IIa*) or partly (Fig. 1, pathway *IIb*). The existence of enzyme – metal – ligand complexes has been demonstrated by NMR (pyruvate kinase),[7] X-ray analysis (carboxypeptidase),[8] and infrared spectroscopy (carbonic anhydrase).[9]

There exist at least two routes[1] for the formation of the enzyme – metal – ligand complexes with a bridge metal:[1]

$$E + M^{n+} \longrightarrow E-M^{n+} \xrightarrow{L} E-M^{n+}-L, \quad (1)$$

$$E + L \longrightarrow E-L \xrightarrow{M^{n+}} E-M^{n+}-L, \quad (2)$$

where L is the ligand.

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A third route of enzyme – metal – ligand complex formation is also possible, where the metal ion non-bound to the enzyme manifests strong affinity for the ligand.

$$M^{n+} + L \longrightarrow M^{n+}-L \xrightarrow{E} E-M^{n+}-L. \quad (3)$$

(3) Complexes with the enzyme as a bridge. Here, the metal ion and the ligand interact with different sites of the enzyme (Fig. 1, pathway *III*). This type of complex is characteristic of glutamine synthetase [10] and some other synthetases.[11, 12]

The catalytic activity of enzymes, which is their most distinctive feature, can vary widely depending on the mode of interaction of metal ions with enzymes. Therefore, the study of effects of metal ions on enzyme activity has both theoretical and practical significance.

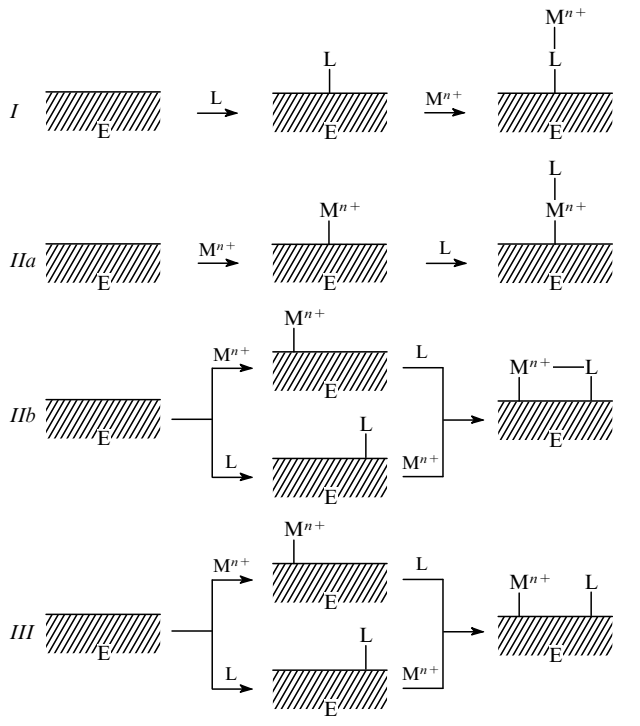


Figure 1. The pathways of interaction of the enzyme (E) with the metal ion (M^{n+}) and the ligand (L).
For *I* – *III*, see text.

This review will not consider metalloenzymes and metal-dependent enzymes. The role of metals in catalytic processes involving these enzymes is the subject of special studies and presents particular interest for biochemistry and medicine (see Refs 13–15). The review summarises the papers devoted mainly to the effect of metal ions on catalytic properties of metal-independent enzymes.

II. The effect of metal ions on the catalytic activity of enzymes

During the last 10–15 years interest in biochemical methods of determination of inorganic toxic agents based on the analysis of properties of native and immobilised enzymes in the presence of metal ions has increased. And the main contribution to the development of this field has been made by Russian scientists. The same conclusion can be made from the analysis of the Proceedings of the 5th International Symposium 'Kinetics in Analytical Chemistry' held in Moscow in 1995, which shows that the chemico-analytical aspect of the effect of metal ions on the catalytic activity of enzymes was mostly covered by reports of Russian investigators.

Most commonly, the catalytic activity of enzymes in the presence of metals is evaluated as changes in the rate of the enzymic reaction. This can be done by measuring the changes in the concentration of the substrate or reaction products with time. A physicochemical parameter (most often, optical density or an ionisation potential) related to the substance concentration is used as the analytical signal.

1. The inhibitory effect of metal ions

Far greater attention has been given to the study of the inhibitory effect of metal ions on the catalytic activity of enzymes than to the activating effect. In most studies, the effect of doubly charged metal cations was examined. In particular, doubly charged ions, like Hg^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, and some others were shown to inhibit bovine ribonuclease A,[16] urease,[17–19] cholinesterases,[20–22] and some others. The I_{50} value (*i.e.*, the inhibitor concentration causing a 50% decrease in the enzyme catalytic activity) and the value of the inhibition constant, K_i, are commonly used for the quantitative estimation of the inhibitory effect.

Thus in order to estimate the inhibitory effect of metal ions (Ag^+, Hg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}) on *St. saprophyticus* urease, the I_{50} values were determined for each of them.[17] The smaller the I_{50} value the stronger the metal inhibits the enzyme. Ag^+ ions ($I_{50} = 8 \times 10^{-6}$ mol litre^{-1}) appeared to be the most effective inhibitor of *St. saprophyticus* urease. According to the strength of their inhibitory effect, these ions can be arranged in the following series: $Ag^+ > Ni^{2+} > Cd^{2+} > Co^{2+} > Hg^{2+} > Cu^{2+} > Zn^{2+} > Pb^{2+}$. The inhibitory action of these ions begins to manifest itself at concentrations from 1×10^{-7} to 2.5×10^{-6} mol litre^{-1}. The maximum inhibitory effect (100%) is observed at the concentrations 5×10^{-4} mol litre^{-1} (Ag^+) and 1×10^{-3} mol litre^{-1} (Hg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}). In the presence of Ni^{2+}, Cd^{2+}, and Co^{2+} ions (10^{-3} mol litre^{-1}) in the reaction medium, complete suppression of the enzyme activity does not occur: urease retains up to 10%–20% of its original activity. The inhibition of urease by the ions assayed is independent of the enzyme concentration and the time of incubation with the solution of a metal salt. The inhibitory effect of all these ions is reversible and is easily eliminated by dilution or desalting of the enzyme on a Sephadex G-25 column. However, when the concentration of Pb^{2+}, Hg^{2+}, Zn^{2+}, and Ag^+ ions in the reaction medium exceeds 10^{-3} mol litre^{-1}, irreversible denaturation of the enzyme takes place. The inhibition, which precluded reactivation of the enzyme, was not observed for Cu^{2+}, Ni^{2+}, Cd^{2+}, and Co^{2+} ions in the whole range of the concentrations studied (1×10^{-7}–1×10^{-3} mol litre^{-1}). Although the authors do not draw such a conclusion, it may be assumed that in this particular case reversible non-specific inhibition takes place.

The values of apparent inhibition constants, K_i, for Mg^{2+}, Co^{2+}, and Mn^{2+} ions, which competitively inhibit the activity of Ca^{2+}-ATPase of skeletal muscle plasma membranes, have been determined.[23] The smaller the K_i value the stronger the effect produced by the inhibitor. It was found that according to their affinity for Ca^{2+}-ATPase, these ions can be arranged in the following order: $Mg^{2+} > Co^{2+} > Mn^{2+}$. The inhibitory effect is a maximum at a Mg^{2+} concentration of 1×10^{-4} mol litre^{-1}. A linear dependence of the degree of inhibition of the enzymic activity on the magnesium concentration in the range of 1×10^{-5}–5×10^{-5} mol litre^{-1} was established. Further increase in the magnesium concentration influenced the inhibition to a lesser degree, *i.e.*, it disturbed the linear dependence.

Mn^{2+} and Co^{2+} ions used at concentrations 1×10^{-5}–1×10^{-4} mol litre^{-1} have no effect on Ca^{2+}-ATPase. Their inhibitory effect becomes apparent only at a concentration of *ca.* 2.5×10^{-4} mol litre^{-1}; a linear dependence of the decrease in the enzyme activity on the cation concentration is observed at concentrations from 2.5×10^{-5} to $(2–3) \times 10^{-3}$ mol litre^{-1}.

The study[16] deserves special mention among other studies concerned with the inhibition of the catalytic activity of enzymes by metal ions. This not only quantitated the effect observed (*i.e.*, the experimental values of inhibition coefficients defined by the author as the ratio of initial rates of substrate cleavage in the presence and in the absence of M^{2+}), but also considered the influence of some other parameters (ionisation potentials, hydration energy, ionic radii of cations) on the magnitude of the inhibitory effect. In the work cited, the effect of Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, and Hg^{2+} on the catalytic activity of bovine RNase A was studied. The inhibitory effect of these cations was found to increase in the series: $Ba^{2+} \approx Sr^{2+} \approx Ca^{2+} < Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Pb^{2+} < Cd^{2+} < Zn^{2+} \ll Cu^{2+} < Hg^{2+}$. It is seen that Hg^{2+}, Cu^{2+}, and Zn^{2+} cations manifest the strongest inhibitory activity; the cations of the iron subgroup (Mn^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+}) are weak inhibitors, whereas the cations Ba^{2+}, Sr^{2+}, and Ca^{2+} do not produce any inhibitory effect at all.

In the author's opinion,[16] the fact that the metal cations do not possess strict selectivity, *i.e.*, that they can interact with functional groups in the enzyme active centre differing in electronic effect, a complex dependence of the mode of enzyme inhibition by the metal cations on their concentration, and their ability to form complexes both with enzymes and the substrates preclude the estimation of the inhibitory activity of metal cations from the inhibition constants.

In order to evaluate the inhibitory strength of cations the author introduces a parameter W, which is equal to the sum of the electrostatic (Z^2/r) and the reduced covalent ($E - \Delta H$) characteristics:

$$W = \frac{Z^2}{r} + (E - \Delta H) ,$$

where Z is the ionic charge, r is the ionic radius, E is the sum of the ionisation potentials for the first and second electrons, and ΔH is the energy of cation hydration.

The higher inhibitory activity of Zn^{2+}, Cu^{2+}, and Hg^{2+} is largely determined by the reduced covalent characteristics. In the cations of the iron subgroup (Mn^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+}), the electrostatic characteristics are comparable with those of Zn^{2+}, Cu^{2+}, and Hg^{2+}, while the values of the reduced covalent characteristics are 2–3 times as low. These cations manifest only weak inhibitory activity. For Ba^{2+}, Ca^{2+}, Mg^{2+}, and Sr^{2+} cations, the values of electrostatic and, particularly, reduced covalent characteristics are smaller than those for Zn^{2+}, Cu^{2+}, and Hg^{2+}. It has been shown experimentally that these cations do not inhibit RNase at the concentrations from 1×10^{-4} to 1×10^{-2} mol litre^{-1}.

The effect of rare-earth elements (REE) (Pr, Nb, Sm, Eu, Tb, Er, Tu, Yb) on the catalytic activity of inorganic pyrophosphatases from bakers yeast and *E. coli* has been studied.[24] Analysis of the dependence of the rate of the indicator reaction on the concentration of REE over the range of $10^{-5}-10^{-1}$ μg ml^{-1} revealed that all these ions inhibited both enzymes; the inhibitory effect of different REE was manifested in different concentration ranges. Praseodymium (Pr^{3+}) appeared to be the most effective inhibitor of pyrophosphatases. The inhibitory effect decreased on going from praseodymium to ytterbium, *i.e.*, with a decrease in the ionic radius of REE. The mechanism of inorganic phosphatase inhibition by praseodymium is consistent with complete competitive inhibition. The value of the inhibition constant, which reflects the stability of the enzyme–inhibitor complex, demonstrates that Pr^{3+} ions form a catalytically inactive complex with pyrophosphatase, which is more stable than that with Eu^{3+} ions.

In other studies,[24–32] only qualitative aspects of the effect of metal ions on the catalytic activity of enzymes have been considered with a mere statement of the existence of the corresponding effect and indicating the range of concentrations where it is manifested.

Cu^{2+} ions decrease the catalytic activity of glutamate dehydrogenase from bovine liver[25] ($I_{50} = 2\times10^{-6}$ mol litre^{-1}). At concentrations of Cu^{2+} equal to 1×10^{-5} mol litre^{-1} and higher, the residual activity makes up 8%–10% of the original. This effect is a result of the direct interaction of the copper cations with glutamate dehydrogenase (the difference absorption spectrum of glutamate dehydrogenase in the presence of Cu^{2+} suggests the formation of an enzyme–metal complex). This inhibition is allosteric. Zn^{2+} ions also inhibit this enzyme. Spectral data point to identical conformational changes in the substrate-binding site induced by Cu^{2+} and Zn^{2+} cations.[25]

Zn^{2+} cations also inhibit poly(ADP-ribose)polymerase from rat brain. At a Zn^{2+} concentration equal to 1.25×10^{-4} mol litre^{-1}, the enzyme is fully suppressed. The changes in the enzyme activity are due to the binding of Zn^{2+} ions with the sulfhydryl groups of polymerase. The inhibitory effect is reversible, since dithiothreitol neutralises the effect of this cation on the polymerase activity.[26]

Pb^{2+} and Cd^{2+}ions ($10^{-3}-10^{-2}$ μg ml^{-1}) inhibit alkaline phosphatase from chicken intestine in the hydrolysis of *p*-nitrophenyl phosphate.[23,27] The decrease in the activity is a maximum at Cd^{2+} concentrations of 1–10 μg ml^{-1} (Ref. 23). Cd^{2+} ions (1×10^{-4} μg ml^{-1}) also inhibit horseradish peroxidase.[27] The catalytic activity of peroxidase is also inhibited by Hg^{2+} cations ($5\times10^{-13}-5\times10^{-8}$ mol litre^{-1}).[28–30] A drop in peroxidase activity is also observed in the presence of Bi^{3+} [$(2-10)\times10^{-4}$ μg ml^{-1}]. At higher Bi^{3+} concentrations, the rate of this reaction remains virtually unchanged.[31]

Protein disulfide reductase from bovine liver catalyses the thiol–disulfide exchange between glutathione and insulin and reactivates randomly oxidised ribonuclease. The activity of this enzyme is inhibited by Cu^{2+} and Hg^{2+} ions.[29]

The effects of Zn^{2+}, Cu^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+} ions on the activity of acetylcholinesterase (AChE) of human erythrocytes have been studied over a broad range of salt concentrations.[20,33] It was found that all the salts in question (with the exception of $MnCl_2$, which has no effect on the AChE activity) are reversible competitive inhibitors of AChE. To a first approximation, the inhibitory effect is directly proportional to the concentration of the corresponding metal. From the pH dependence of the AChE inhibition by the salts it was concluded that the AChE activity is inhibited by hydroxo complexes of the MOH^+ type.

Cu^{2+} and Cd^{2+} ions suppress the catalytic activity of cholinesterase (ChE) isolated from pea sprouts.[22] Co^{2+} and Al^{3+} ions also manifest inhibitory activity. According to another report,[21] Ca^{2+} and Mn^{2+} used at concentrations of $1\times10^{-3}-1\times10^{-2}$ mol litre^{-1} inhibit ChE from kidney bean roots, whereas Mg^{2+} has no effect on the enzyme.

Calcium occupies a special position among metals, since it manifests an activating rather than inhibitory effect.[34] However, it can also inhibit certain enzymes, *e.g.*, yeast hexokinase[30] and alkaline phosphatase.[27]

It has been established that calcium ions taken at a 10–100-fold excess over praseodymium enhance the inhibitory effect of the latter. The inhibitory action of calcium[24] is ascribed to the fact that its ionic radius is similar to those of REE and thus it can exert a similar effect on the enzyme.

Ca^{2+} cations markedly decrease the activity of hexokinase.[35] The catalytic activity of alkaline phosphatase from chicken intestine is also decreased in the presence of calcium ions ($10^{-3}-1$ μg ml^{-1}). It should be noted that the inhibitory effect of calcium on the enzyme decreases with an increase in the ion concentration.[27]

Recently, some papers have been published, which deal with the study of the catalytic activity and properties of immobilised enzymes.[18,19,36–38] Naturally, metal cations also change the catalytic activity of immobilised enzymes.

Urease immobilised on a solid carrier (a microporous styrene–divinylbenzene copolymer) is inhibited by ions of toxic metals. The inhibitory effect of Fe^{2+} manifests itself even at a concentration of $10^{-10}-10^{-9}$ mol litre^{-1}, whereas that of most ions, at 10^{-8} mol litre^{-1}. With respect to the strength of their inhibitory action, the metal ions can be arranged in the following series:[18,19] $Ag^+ > Hg^{2+} > Fe^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Al^{3+} > Mg^{2+} > Cd^{2+} > Pb^{2+}$. As noted above, Ag^+ is the strongest and Pb^{2+} is the weakest inhibitor of native urease.[17] At the same time, the series of other metals for the native urease (see above) differs from that for the immobilised enzyme due to the changes caused by enzyme immobilisation.

A number of papers have been devoted to the effects of Zn^{2+}, Fe^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Tl^+, Bi^{3+}, Cd^{2+}, Cu^{2+}, Cr^{3+}, Hf^{4+}, Ti^{4+}, and Zr^{4+} ions on the catalytic activity of cholinesterase immobilised by incorporation into cellulose nitrate films.[36–38] All these metals inhibit the immobilised enzyme. The greatest inactivating effect is produced by Cu^{2+}. The minimum Cu^{2+} concentration (c_{min}), which causes a statistically significant decrease in the catalytic activity of immobilised ChE, is 4×10^{-10} mol litre^{-1}. A similar change in the catalytic activity in the presence of other ions is observed at the following concentrations:[36–38]

Ion	Zr (IV)	Ti (IV)	Nb (V)	Hf (V)	Ta (V)
c /mol litre^{-1}	5×10^{-10}	8×10^{-10}	2×10^{-9}	3×10^{-9}	5×10^{-9}

Ion	Cr (III)	Pb (II)	Fe (III)	Cd (II)
c /mol litre^{-1}	5×10^{-8}	5×10^{-7}	1×10^{-6}	1×10^{-5}

The inhibitory effect also depends on the catalytic activity of the enzyme itself. Thus, for ChE immobilised in a cellulose nitrate matrix with a specific activity of 1.12±0.04 μmol min^{-1} cm^{-2}, c_{min} for Cu^{2+} is 4×10^{-10} mol litre^{-1}, whereas for the samples with a threefold lower specific activity (0.33±0.02 μmol min^{-1} cm^{-2}), it is equal to 1×10^{-9} mol litre^{-1} (Ref. 38).

Solutions of cysteine and EDTA restore completely the catalytic activity of immobilised ChE after the action of heavy metal ions. However, only 70% of totally inhibited ChE is observed in the case of Pb^{2+}. This is probably due to the interaction of Pb^{2+} with the histidine residue of the active centre of ChE, especially in the presence of high ($\sim 10^{-4}$ mol litre^{-1}) concentrations of Pb^{2+} (Ref. 36).

The mechanism of the inhibitory effect of metal ions on the catalytic activity of enzymes is rather difficult to establish. The metal can compete with the substrate for the binding sites in the active centre, it can also interact with different groups in the protein molecule outside the active centre but influencing the catalytic functions of the enzyme,[39] *i.e.*, it can bind to the allosteric centre of the enzyme.

It is believed that the effect of metal ions on the enzyme molecule can be due to the formation of thiolates with both

cysteine, which is a constituent of the enzyme, and products of cleavage of disulfide bridges.[40] If the cysteine residue is incorporated into the active centre of the enzyme or is responsible for the maintenance of the appropriate conformation of the protein molecule, the modification of its thiol group inactivates the enzyme. In addition, heavy metal ions can catalyse the oxidation of thiols to disulfides:[41]

$$RSH + O_2 + R'SH \longrightarrow R-S-S-R' + H_2O_2.$$

Most commonly, the inhibitory effect of metal ions is ascribed to their ability to form coordinate bonds.[41] As is known,[42] the nucleophilic residues of some amino acids incorporated into the active centres of enzymes, can form coordinate bonds with heavy metal ions. For example, histidine, which is a constituent of the esterase region of the active centre of ChE, can interact with metal ions.[36] Some authors explain the difference in the inhibitory action of various metal ions by the difference in the ionic radii of cations.[16, 29, 36]

2. The activating effect of metal cations

Metals can not only inhibit but also enhance the catalytic activity of enzymes. Most often, the activating effect is produced by ions of rare-earth metals. It should be noted that the processes related to the activation of enzymes with metal ions have been documented in far fewer publications than those on the inhibition processes. Unfortunately, in the case of studies of the inhibitory effect, most authors give only a qualitative outline of the activation process.[43–56] without quantitative estimates, which makes it difficult to compare the published data.

The activating effect of Ca^{2+} cations on mitochondrial glycerol-3-phosphate dehydrogenase has been studied.[43] Ca^{2+} cations are not selective activators. They interact with both electron-donor amino acid residues and those nitrogen atoms of the peptide bonds that are not necessarily incorporated into the enzyme active centre. It thus follows that not all Ca^{2+} cations that interact with the enzyme play the same role in the maintenance of its activity. Some of them that interact with the amino acid residues remote from the enzyme active centre not only are devoid of the activation effect, but, conversely, produce an inhibitory effect.

Analysis of literature data reveals that the simultaneous presence of several metal cations, even of the same nature (Ca, Mg), has an ambiguous effect on the activity of the enzyme under study: one of the ions can either enhance the effect of the other ion (synergism) or attenuate it.

Most often, the synergistic effect is produced by Ca^{2+} and Mg^{2+} ions.[34] For example, even a short-term preincubation of phosphorylase kinase with these ions caused a sevenfold increase in the catalytic activity. It is believed that the simultaneous presence of Ca^{2+} and Mg^{2+} favours the interaction of phosphorylase kinase with glycogen.[34]

Ca^{2+} and Mg^{2+} ions also activate myofibrillar creatine kinase from rat skeletal and cardiac muscles. Calcium activates the enzyme to a lesser degree than magnesium. In the presence of Mg^{2+} ions, calcium inhibits creatine kinase. Presumably, creatine kinase has different binding sites for Ca^{2+} and Mg^{2+} ions, since the latter does not eliminate the inhibitory effect of calcium even when used in a 100-fold excess.[44]

Ca^{2+} and Mg^{2+} ions activate myometrium actomyosin ATPase. As the Ca^{2+} concentration is increased $(0.01-5)\times10^{-3}$ mol litre^{-1} in a Mg^{2+}-free medium or after addition of Mg^{2+} (1×10^{-3} mol litre^{-1}), the ATPase activity is noticeably increased and then reaches a plateau.[39]

A change in the Mg^{2+} concentration from 1×10^{-5} to 5×10^{-3} mol litre^{-1} in a Ca^{2+}-free medium increases the activity of ATPase in the region of low concentrations after which it reaches a plateau. Significant induction of the ATP-hydrolase reaction is observed after addition of the physiological concentration of Ca^{2+} (1×10^{-3} mol litre^{-1}) to the incubation medium. Ca^{2+} ions are more effective activators of the reaction of ATP hydrolysis.[46]

The effect of cations of alkaline and alkaline-earth metals on the activity of native ChE[21, 46] and immobilised butyrylcholinesterase[36] has been studied. Na^+, K^+, and Mg^{2+} ions stimulate the cholinesterase activity in pea sprouts by 30%–60%.[21, 22] The catalytic activity of immobilised ChE increases in the presence of Na^+, Mg^{2+}, and Mn^{2+} ions in the concentration range of $8\times10^{-4}-2\times10^{-2}$ mol litre^{-1}.[36] Presumably, this is due to the presence of an anionic group near the active centre of ChE, which prevents the interaction of the enzyme with the substrate. Introduction of these metal salts results in binding of this group by ions of ChE-activator metals, which creates the most favourable conditions for the formation of the Michaelis complex. It was found that the larger the radius and the charge on the activator metal the lower the concentration of this metal ion needed to block the anionic group of ChE.

Ca^{2+} cations activate butyrylcholinesterase.[47–49] The maximum rate of the reaction is increased in the presence of Ca^{2+}, whereas the Michaelis constant remains practically unchanged. Apparently, Ca^{2+} ions do not compete with the substrate for the binding sites, since the activation cannot be eliminated by an excess of acetylcholine. It is believed that Ca^{2+} exerts an allosteric effect as it binds with specific sites.[50, 51] Co^{2+}, Ni^{2+}, and Mn^{2+} ions do not influence the acetylcholine hydrolysis.[47]

Immobilised ChE is activated by microquantities of heavy metals, such as Pb, Hg, Cd, and Tl used generally at concentrations below $10^{-7}-10^{-6}$ mol litre^{-1}.[52, 53] Some platinum metals (Pt^{2+}, Rh^{3+}) also activate the immobilised ChE.[54, 55]

It should be noted that the inhibitory effect correlates linearly with the concentration of the effector.[27–31, 37, 38] As to the activation, this process demonstrates a more complex dependence on the concentration of metal ions.[35, 36] First of all, an increase in the catalytic activity is observed over a small range of concentrations that differ usually by not more than a factor of 10–15 (Fig. 2). The maximum catalytic activity is manifested sometimes only at a definite concentration of the effector (Fig. 2*b*). In some cases, a small plateau is observed (Fig. 2*a*). A deviation from the optimum concentration results in a decrease of the enzyme catalytic activity towards the control level.

Among studies concerned with enzyme activation, that dealing with the mechanism of the activating effects of Mg^{2+} and Co^{2+} ions on glucose isomerase of *Actinomyces olivocinereus* 154 stands separately.[57] In this study, the main kinetic constants have been calculated and quantitative estimates of the activating capacity of these cations (the activation constants) have been given. It was found that the activating effect of Mg^{2+} ions is much stronger than that of Co^{2+}. The maximum activating effect of Mg^{2+} ions is observed at a concentration $(2.0-2.5)\times10^{-2}$ mol litre^{-1}, that of Co^{2+} ions, at $(2.5-5)\times10^{-4}$ mol litre^{-1}. Further increase in the concentrations of Mg^{2+} and Co^{2+} ions leads to the inhibition of glucose isomerase; for Co^{2+} ions, this phenomenon is more strongly pronounced and manifests itself even at a concentration of 1.25×10^{-3} mol litre^{-1}.

Studies of the activating effect of Mg^{2+} and Co^{2+} ions added to the reaction mixture simultaneously revealed a complex dependence of the enzyme activation on both the absolute concentrations of these metal ions and their ratio. The maximum activity was observed at a Mg^{2+} ion concentration of 2×10^{-5} mol litre^{-1} and that of Co^{2+} at 5×10^{-3} mol litre^{-1}. It should be noted that for Mg^{2+} the optimum concentrations of this activator ion used in a mixture were slightly lower than when it was added separately, whereas for Co^{2+} ions, they were tenfold higher.

Mg^{2+} and Co^{2+} ions bind to different sites on glucose isomerase and influence its activity differently.[57] Therefore, the mechanism of activation of the glucose isomerisation reaction by these ions is also different. The activation with Mg^{2+} ions is non-competitive, while that with Co^{2+} ions is synergistic; when Mg^{2+} and Co^{2+} ions are used simultaneously, the activation is of a mixed type. In the non-competitive activation, glucose and Mg^{2+} ions bind to the enzyme independently of each other to form an

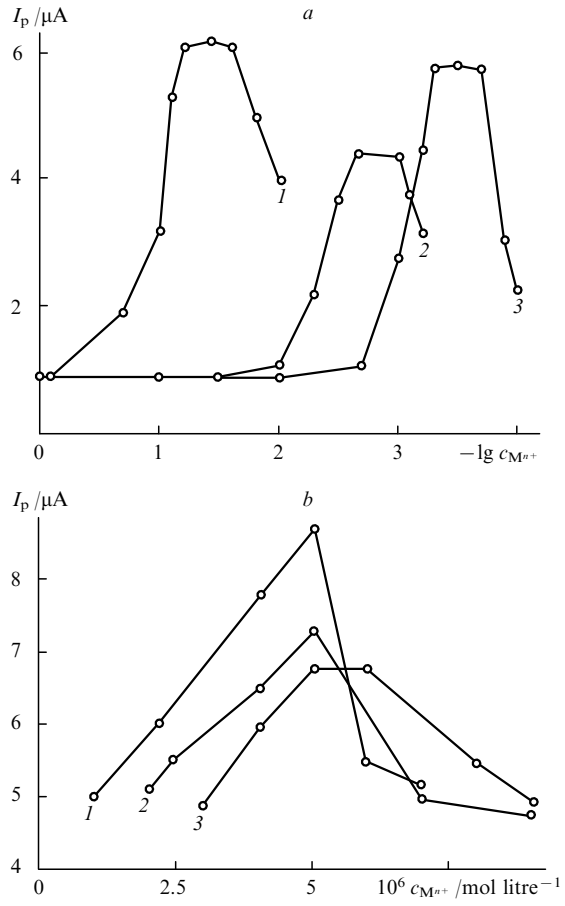


Figure 2. Dependence of the analytical signal (electric current at a potential of -0.55 V) on the concentration of metal ions (pH 9.05; the concentration of butyrylthiocholine iodide, 0.6 mg ml^{-1}).
Specific activity of immobilised ChE is 0.35 μmol min^{-1} cm^{-2} (*a*) and 1.17 μmol min^{-1} cm^{-2} (*b*).
(*a*): (*1*) — Mn (II), (*2*) — Mg, (*3*) — Na; (*b*): (*1*) — Ca, (*2*) — Sr, (*3*) — Ba.

active bridge-like ternary complex (Fig. 1, pathway *II*). The value of the activation constant is very low (0.002), which points to a high stability of an active ternary complex ($Mg^{2+}-E-S$) and the activated enzyme ($Mg^{2+}-E$) and to a small rate constant for their dissociation into the original enzyme and the enzyme–substrate complex (ES). Thus, in the activation of glucose isomerase with Mg^{2+}, the major part of the enzyme is bound into the ternary complex $Mg^{2+}-E-S$. As has been mentioned above, the activation with Co^{2+} ions is synergistic. Co^{2+} ions bind to the glucose isomerase molecule and increase the enzyme affinity for glucose. In addition, the ES complex interacts with Co^{2+} ions much faster than the free enzyme. The authors suggest that the main role of Co^{2+} ions consists in conferring a conformation that is resistant to thermal inactivation on the enzyme quaternary structure rather than in the activation of the enzyme. Moreover, this conformation facilitates the attachment of glucose even in the absence of Mg^{2+} ions and accelerates the formation of a bridge-like ternary complex in their presence, as a result of which the rate of isomerisation increases. This is corroborated by experiments with simultaneous addition of Mg^{2+} and Co^{2+} ions to the reaction mixture.[57]

Attempts have been undertaken[56, 58] to characterise quantitatively the processes of activation of immobilised ChE in the presence of alkaline-earth metal ions on the basis of voltammetric measurements. The results of these studies indicate that the activation is controlled by many factors, such as substrate concentration, pH, activity of the immobilised enzyme (*i.e.*, the type of immobilisation), activator concentration, incubation time, and the order in which the substrate and the effector act on immobilised ChE. The maximum activating effect was observed at pH 8.95–9.05. The lower the catalytic activity of immobilised ChE the higher the concentration of metal ions needed for the manifestation of their activating effect. Thus immobilisation according to Nikolskaya [33] in which incorporation of the enzyme into a cellulose nitrate matrix was performed together with treatment with a bifunctional reagent (glutaraldehyde) gave immobilised ChE with a catalytic activity about 3–4 times lower than that obtained by immobilisation of the enzyme followed by treatment with a bifunctional reagent. In order to increase the catalytic activity of these samples, it is necessary to use high concentrations of the activators ($6\times10^{-3}-6\times10^{-4}$ mol litre^{-1} Ca^{2+}).[37] To verify these effects, the authors carried out a radiochemical analysis with ^{85}Sr. The results of this study suggest that under optimum conditions (pH 9.05, 5×10^{-6} mol litre^{-1} Sr^{2+}, substrate concentration, 2×10^{-3} mol litre^{-1}) alkaline-earth metal ions change the catalytic activity of immobilised ChE in the order: $Ca^{2+} > Sr^{2+} > Ba^{2+}$, which is consistent with their ionic radii. Similar correlation between the ion radii and their activating effect was observed earlier for alkali metal ions.[36] As the substrate concentration is increased from 2×10^{-3} to 4×10^{-3} mol litre^{-1}, the type of activation changed from associative to pseudo- and then to two-parametrically disbalanced activation. The activation constants for Ca^{2+} ions are $(3.7\pm0.3)\times10^{-6}$ mol litre^{-1} at low [$(1.5-3)\times10^{-3}$ mol litre^{-1})] and $(1.8\pm0.2)\times10^{-8}$ mol litre^{-1} at higher (4×10^{-3} mol litre^{-1}) substrate concentrations; that for Ba^{2+} ions is $(8.40\pm0.3)\times10^{-6}$ mol litre^{-1}. The experimental values of the activation constants correlate with a stronger activating effect of Ca^{2+} ions (the catalytic activity of immobilised ChE is increased more strongly in the presence of Ca^{2+}).

3. The dependence of the mode of action of metal ions on their concentration

It is well known that the same metals can increase or decrease the catalytic activity of enzymes depending on their concentration.

Thus Cu^{2+} ions used in low concentrations stimulate the activity of purified poly(ADP-ribose)polymerase. At a Cu^{2+} concentration of 5×10^{-6} mol litre^{-1}, the polymerase activity is increased 1.4-fold. Further increase in the Cu^{2+} concentration inhibits the enzyme activity and with 5×10^{-5} mol litre^{-1} Cu^{2+} the polymerase activity drops down to zero. Poly(ADP-ribose)-polymerase contains sites with different affinity for Cu^{2+} cations. At low Cu^{2+} concentrations, the binding of these ions to the high-affinity sites of the enzyme increases the polymerase activity, whereas at high Cu^{2+} concentrations, the binding may also occur to sites with a lower affinity for the metal, which results in the inhibition of the enzyme activity.[26]

Mg^{2+} ions ($10^{-3}-1$ μg ml^{-1}) inhibit alkaline phosphatase from chicken intestine. They strongly activate the enzyme over the concentration range 1–100 μg ml^{-1}. Aluminium ions slightly inhibit the enzyme when used at the concentrations of $10^{-3}-1$ μg ml^{-1} but strongly activate it at the concentrations of 1–10 μg ml^{-1} (Ref. 27).

Low concentrations of Mn^{2+}, Ni^{2+}, and Co^{2+} ions activate muscle pyruvate kinase, whereas higher concentrations inhibit the enzyme. Ca^{2+} ions ($4\times10^{-4}-2\times10^{-4}$ mol litre^{-1}) inhibit liver glutamate dehydrogenase and activate it at concentrations above 4×10^{-4} mol litre^{-1}. At the same time, Mg^{2+} ions inhibit glutamate dehydrogenase only in the concentration range of $(2-4)\times10^{-4}$ mol litre^{-1}. Higher and lower concentrations of Mg^{2+} activate this enzyme. Some phosphatases are activated by low concentrations of Mg^{2+} (up to 5×10^{-3} mol litre^{-1}), whereas higher concentrations of this ion cause their inhibition.[34]

The changes in the properties of enzymes in the presence of some metal ions are summarised in Table 1.

Almost all investigators emphasise that the effect of metal ions on the activity of ChE is of dual character: activation at small concentrations and inhibition at high concentrations.[47] Even such cations as Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} do not increase the catalytic activity of ChE when used at high concentrations.[56]

Table 1. The effect of some metal ions on the catalytic activity of enzymes.

Enzyme	Metal ion	Effect	c_{eff} [a] /mol litre^{-1}	Ref.
Ca^{2+}-ATPase	Mg^{2+}, Co^{2+}	inhibitor	$1\times10^{-5}-1\times10^{-3}$	23
EC 3.6.1.3.	Mn^{2+}	"	$1\times10^{-5}-1\times10^{-3}$	23
ATPase	Ca^{2+},	activator	$1\times10^{-5}-5\times10^{-3}$	45
EC 3.6.1.8.	Mg^{2+}	"	$1\times10^{-5}-5\times10^{-3}$	45
Hexokinase EC 2.7.1.1.	Ca^{2+}	inhibitor	$1\times10^{-3}-5\times10^{-2}$	35
Glycerol-3-phosphate dehydrogenase EC 1.1.1.8	Ca^{2+}	activator	$1\times10^{-6}-1\times10^{-5}$	43
Glutamate	Cu^{2+},	inhibitor	$1\times10^{-6}-1\times10^{-4}$	25
dehydrogenase	Zn^{2+}	"	$1\times10^{-6}-1\times10^{-4}$	25
EC 1.4.1.4.				
Glucose	Mg^{2+},	activator	$1\times10^{-3}-9\times10^{-1}$	57
isomerase	Co^{2+}	"	$1\times10^{-3}-9\times10^{-1}$	57
EC 5.3.1.18.				
Creatine kinase	Ca^{2+},	"	$1\times10^{-3}-1\times10^{-2}$	44
EC 2.7.3.2.	Mg^{2+}	"	$1\times10^{-3}-1\times10^{-2}$	44
Inorganic	Pr, Nb, Sm	inhibitor	$1\times10^{-5}-1\times10^{-1}$	24
pyrophosphatase	Eu, Tb, Er	"	$1\times10^{-5}-1\times10^{-1}$	24
EC 3.6.1.1.	Tu, Yb	"	$1\times10^{-5}-1\times10^{-1}$	24
Peroxidase	Hg^{2+}	"	$5\times10^{-13}-5\times10^{-8}$	28
EC 1.11.1.7.	Bi^{3+}	"	$1\times10^{-5}-2\times10^{-4}$	31
Poly(ADP-ribose)poly-merase	Zn^{2+}	"	$2\times10^{-5}-2\times10^{-4}$	26
	Cu^{2+}	"	$1\times10^{-5}-5\times10^{-5}$	26
	Cu^{2+}	activator	$1\times10^{-6}-1\times10^{-5}$	26
EC 2.4.2.30				
Ribonuclease	Ba^{2+}, Sr^{2+}	no influence	$1\times10^{-4}-1\times10^{-2}$	16
EC 3.1.4.8.	Ca^{2+}	"	$1\times10^{-4}-1\times10^{-2}$	16
	Hg^{2+}, Cu^{2+}	inhibitor	$1\times10^{-4}-1\times10^{-2}$	16
	Zn^{2+}, Mn^{2+}	"	$1\times10^{-4}-1\times10^{-2}$	16
	Fe^{2+}, Co^{2+}	"	$1\times10^{-4}-1\times10^{-2}$	16
	Ni^{2+}	"	$1\times10^{-4}-1\times10^{-2}$	16
Urease	Ag^{+}, Hg^{2+}	"	$1\times10^{-7}-1\times10^{-3}$	17
EC 3.5.1.5.	Cu^{2+}, Zn^{2+}	"	$1\times10^{-7}-1\times10^{-3}$	17
	Pb^{2+}, Ni^{2+}	"	$1\times10^{-7}-1\times10^{-3}$	17
	Cd^{2+}, Co^{2+}	"	$1\times10^{-7}-1\times10^{-3}$	17
Cholinesterase	Pb^{2+}	"	$5\times10^{-7}-1\times10^{-3}$	36
EC 3.1.1.8.	Pb^{2+}	activator	$1\times10^{-9}-1\times10^{-7}$	53
	Cd^{2+}	inhibitor	$1\times10^{-6}-1\times10^{-3}$	37
	Cd^{2+}	activator	$5\times10^{-9}-8\times10^{-7}$	53
	Hg^{2+}	inhibitor	$5\times10^{-5}-1\times10^{-3}$	36
	Hg^{2+}	activator	$1\times10^{-12}-1\times10^{-6}$	53
	Tl^{+}	inhibitor	$5\times10^{-5}-1\times10^{-3}$	37
	Tl^{+}	activator	$1\times10^{-11}-1\times10^{-5}$	53
	Ca^{2+}, Sr^{2+}	"	$1\times10^{-6}-1\times10^{-5}$	56
	Ba^{2+}	"	$1\times10^{-6}-1\times10^{-5}$	56
Alkaline	Ca^{2+}	inhibitor	$2\times10^{-8}-3\times10^{-5}$	27
phosphatase	Ba^{2+}	"	$7\times10^{-9}-7\times10^{-7}$	27
EC 3.1.3.1.	Cd^{2+}	"	$9\times10^{-9}-9\times10^{-4}$	27
	Pb^{2+}	"	$5\times10^{-9}-5\times10^{-4}$	27
	Mg^{2+}	"	$4\times10^{-8}-4\times10^{-5}$	27
	Mg^{2+}	activator	$4\times10^{-5}-4\times10^{-3}$	27

[a] c_{eff} is the concentration range where the effect is observed.

Pb^{2+}, Hg^{2+}, Cd^{2+}, and Tl^{+} ions activate ChE at concentrations below $10^{-7}-10^{-6}$ mol litre^{-1} and inhibit the enzyme at higher concentrations.[36,37,52,53] Most authors attribute the activating effect of cations to the possible formation of the ternary complex E–M–S (Fig. 1, pathway *II*),[1,58] which provides conformationally more favourable conditions for the enzymic reaction. The inhibitory effect of high concentrations of metal ions is assigned to the competitive reaction between metal and substrate cations (if the substrate bears a positive charge) for the active centre of the enzyme. At high salt concentrations, the active centre of the molecule can be occupied by metal ions, thus being inaccessible to the substrate cations. As a result, the catalytic activity of both native and immobilised ChE may decrease.[36,59] According to other authors,[60] the effect of metal ions on the enzyme activity is due to changes in the substrate solubility at high salt concentrations.

Obviously, the effect of metal ions on enzymes depends on many factors. It is therefore difficult and usually impossible to identify the determining factor. In particular, the effect of metals on the catalytic acivity of enzymes depends on the nature of the metal, its size, charge, ionisation potential, the nature of the enzyme, its state (native or immobilised), conditions of the enzymic reaction, *etc*. Most commonly, the effect of metal ions on the enzyme activity is associated with their ability to form enzyme–substrate complexes, which may participate in the catalytic process by facilitating the transfer of electrons, to stabilise the active conformation of the enzyme or association of its subunits, and to act as an 'anchor' group without being directly involved in the catalytic act. These and other effects may also occur in combination. Unfortunately, the majority of authors give only qualitative characteristics of changes in the enzymic activity, which naturally hampers comparative analysis of the experimental data, and only very few try to estimate these effects quantitatively and get an insight into the mechanism of action of metal ions. These studies have received the greatest attention in the present review.

III. Changes in the catalytic activity of enzymes in the presence of metal ions: an analytical aspect

Over a long period of time, investigators have merely noted the effects of metal ions on the catalytic activity of enzymes, but these effects did not find practical application. As the bulk of experimental data have been accumulated, the analytical application of these effects became possible. The studies on this subject covering the period of 1949–1979 have been summarised in a review.[61]

One of the first studies by Russian authors on the application of the effects of metals on enzymes for analytical purposes was that by Dolmanova *et al.*[28] in which the authors offered a highly selective procedure for the quantitation of mercury, which was based on the inhibitory effect of mercury on horseradish peroxidase [the detection limit is $(1.0\pm0.5)\times10^{-5}$ μg ml^{-1}]. Further investigations[29] in this field were directed to the use of various substrates and the optimisation of conditions for enzymic reactions, which made it possible to lower the detection limit to 3.0×10^{-7} μg ml^{-1}. Bismuth and cadmium ions interfere with the determination. A procedure for quantitation of mercury was proposed[30] (in which the lower limit of concentrations is 1×10^{-5} μg ml^{-1}), which differed from the standard ones by being time-saving (the analysis lasted for 30 minutes instead of 4 hours) and which permitted determination of mercury in the presence of relatively high ($\geqslant 1$ μg ml^{-1}) concentrations of Fe^{3+}. Iron is masked by tartaric acid, which has no effect on the catalytic activity of peroxidase under the given experimental conditions and at selected concentrations. Quantitation of mercury in natural water is carried out using the method of additives, which makes it possible to estimate the effect of other components of the matrix (particularly that of Fe^{3+}) without masking.

The inhibitory effect of Bi^{3+} on the catalytic activity of horseradish peroxidase was used as the basis for the kinetic method used for its quantitation. The lower limit of concentrations that can be determined is 2×10^{-4} μg ml^{-1}. This assay is not hampered by a 1000-fold excess of Ca, Mg, Zn, Ni, and Al, by

100-fold excess of Pb and Ag, and by a 10-fold excess of Cd. The presence of comparable amounts of Hg^{2+} interferes with the analysis.[31,62]

A highly sensitive ($c_{min} = 8\times10^{-6}-4\times10^{-4}$ μg ml^{-1}), selective enzymic method[24] for the quantitation of REE is based on their inhibitory effect on the catalytic activity of bakers yeast and *E. coli* pyrophosphatases. The interference of Co^{2+}, Ni^{2+}, and Mn^{2+} ions was neutralised by introducing an excess of magnesium into the indicator reaction. The addition of 5×10^{-3} mol litre^{-1} of magnesium in the presence of 5×10^{-5} μg ml^{-1} of praseodymium and of $5\times10^{-5}-5\times10^{-3}$ μg ml^{-1} of manganese, cobalt, and nickel to the system eliminates their interference; this magnesium concentration has no effect on the inhibitory effect of praseodymium. The interference of calcium ions was eliminated by masking it with tartrate.

The high sensitivity of enzymic methods makes it possible to determine many components of various waters in small samples and without preliminary concentration at the maximum permissible concentration (MPC) level. For example, metal ions like Cu, Co, Ni, Fe, Mn, Cr, Hg, Cd, Bi, Pb, and Zn can be detected at concentrations of 1–100 pg ml^{-1} on the basis of their inhibitory effect on horseradish peroxidase and alkaline phosphatase.[63]

It was proposed to use enzymic methods in the study of formation of hydroxo complexes. The stability constants of hydroxo complexes, K_{MOH^+}, have been calculated for zinc, copper, iron, cobalt, and nickel.[20] Analytical methods based on the use of enzymes also permit measurement of the equilibrium concentrations of hydroxide ions and of ions of some *d*-block elements in complex heterogenous systems and the study of salt hydrolysis with a high degree of sensitivity and selectivity unattainable by the majority of other methods.[34]

The use of native enzymes for some specific assays has a number of disadvantages, such as the instability of enzymes on storage and under the influence of various factors (*e.g.*, heat treatment) and the impossibility to separate enzymes from the reagents and the reaction products. A contemporary approach to the solution of this task consists in the wide use of immobilised enzymes. Immobilisation favours the stabilisation of enzymes on long-term storage and increases their resistance to external factors. The possibility of multiple use of enzymes reduces considerably the cost of analysis.

The use of immobilised enzymes in metal ion assays has been reviewed.[64] Immobilisation of enzymes for determination of metal ions entails their incorporation into various membranes or binding to glass beads. Polymeric films with immobilised enzymes incorporated in them are now often used as membranes for biochemical sensors.

Thus an amperometric biochemical sensor for determination of metal ions has been constructed on the basis of ChE immobilised by incorporation into cellulose nitrate films.[36–38] A method for assaying Cr^{3+}, Ti^{4+}, Zr^{4+}, and Hf^{4+} ($1\times10^{-10}-1\times10^{-5}$ mol litre^{-1}) has been developed, which is based on the inhibitory effect of heavy metal ions on the catalytic activity of immobilised cholinesterase.[38]

It was suggested [65] that Cu^{2+} be determined using an enzymic electrode containing glucose oxidase; it is based on the inhibitory effect of these cations on the enzyme in the concentration range of $2.5\times10^{-4}-5\times10^{-3}$ mol litre^{-1}.

Recently, much attention has been paid to semiquantitative test-methods, which allow rapid, simple, and reliable determination of concentrations of toxic substances at the MPC level. The use of immobilised enzymes for the elaboration of these methods is also very promising.

A test-method for the determination of Hg^{2+} using peroxidase immobilised as a solid solution in chitosan has been described.[66] The assay is based on the inhibitory effect of mercury on the oxidation of *o*-dianisine, *o*-phenylenediamine, and 3,3′,5,5′-tetramethylbenzidine with hydrogen peroxide in the presence of thiourea catalysed by immobilised peroxidase. The change in the colour of the reaction solution due to the formation of red or orange products of enzymic oxidation allows visual monitoring of the reaction rate. The lower limits of mercury concentrations that can be determined in these reactions are 1×10^{-5}, 5×10^{-5}, and 1×10^{-7} μg ml^{-1}, respectively. This method is noted for selectivity (the assays are hampered only by a 10^5-fold excess of cadmium and bismuth), rapidity (the analysis is completed within 15 minutes), simplicity, and low cost.

Enzymic methods for assaying metal ions are characterised by high sensitivity and in some cases, high selectivity.[27] However, most of these assays are not sufficiently selective. A combination of high sensitivity of biochemical sensors based on immobilised enzymes with traditional methods of masking makes it possible to solve complex analytical problems. Thus, enzymes act as a new generation of analytical reagents. The use of biosensors for quantitation of metal ions is also a promising approach because it exploits the conditions modelling individual functions of a living organism. The elaboration of novel measuring tools including biosensors, especially those based on highly sensitive methods of registration, considerably extends the possibilities of evaluation of the effects of metal ions on the catalytic activity of enzymes. It may be assumed that in many cases the concentration range of inhibition or activation for a definite 'metal–enzyme' pair could not be determined because of the limited potentialities of the recording systems. In addition, in the case of specific assays, allowance should be made for the ionic composition of the medium and the composition of the buffer solutions used for each assay. Buffer solutions must be selected in such a way that their ionic composition ensures the maximum catalytic activity of the enzyme. This provides the conditions for the generation of a maximum analytical signal, which in turn extends the analytical capabilities of enzymic assays of various effectors including metal ions.[37,57]

IV. Conclusion

The above analysis of literature data shows that the effect of metal ions on the catalytic activity of enzymes is still a timely problem. At the same time, special mention should be made of some recent specific features of these studies. Thus in the 1960–70's interest in these studies was mostly biological or medical, while in the last decade interest has been shifted towards the chemical aspects of the problem. This is primarily due to the pollution of the biosphere with various metals above the permissible level and secondly, due to the possibility of their estimation with the help of enzyme preparations possessing higher sensitivity and, in some cases, selectivity. Two main trends in these studies can be isolated: quantitative assay of inhibitors or activators from the magnitude of the induced effect and the use of the activating effect of metal ions for increasing the sensitivity of assays for a wide range of compounds of various natures.

An overview of current literature demonstrates that it is difficult to predict *a priori* how one or another metal will influence the enzyme. This depends not only on the nature of the metal and the enzyme, but also on other specific conditions. The most important of those are the ionic radius and the concentration of the metal. One should not consider the problem of interaction of metal ions with the enzyme separately from the pH of the solution and the presence or the absence of the substrate. The activating or inhibitory effect of metal ions must be considered only with allowance for these factors.

Unfortunately, the majority of authors restrict themselves currently to the qualitative description of effects of metal ions on the catalytic activity of enzymes, while very few papers deal with quantitative estimates and analysis of the mechanism of interaction between metals and enzymes. It may be supposed, however, that the number of such publications will be increasing with the development of highly sensitive methods of registration.

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