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Metal coordination compounds: Models for biological and biochemical systems and their potential analytical applications *

CHANAN SINGH AND GYAN P. SRIVASTAVA
Biochemistry Division, Central Drug Research Institute, Lucknow 226 001, India.

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Abstract

In this review, an attempt is made to present comprehensively several new and interesting properties of analytical biological and cytochemical significance of metal coordination compounds with a particular stress towards their fundamental and/or practical implications. With the exploration of newer aspects of metal coordination compounds as models of positively-charged biomolecules, many a new area of research pertaining to inorganic, physical and analytical biochemistry has been opened up and as a result new information has emerged on various aspects of bile salts, chelators, dyes, drugs, inorganic iso-and hetero-polyacids, biopolymers, etc. This review is supported with 114 references.

Key words: Metal coordination compounds, biomolecular interactions, macroionic complexation, metachromasia, inorganic polyphosphates, acid mucopolysaccharides, bile salts, polyanions, polycations, nucleic acid protection, dyes, substrate immobilization, urea, dimethylsulphoxide.

1. Introduction

The living systems are dependent not only upon organic but also upon the inorganic moieties and biomolecules of diverse structures and compositions which in turn are coupled with a balanced interplay of various physico-chemical forces of the life processes. This has aroused an intense curiosity amongst the investigators to understand the deeper implications of the role of inorganic chemistry in living systems[1-4]. A closer examination of the earlier literature in biochemistry and physiology shows that inorganic compounds have been profitably employed as models[5-8] for understanding and unravelling intricacies of various biochemical and biological problems associated with the life processes. Accordingly, the present article is not intended to cover the vast area of metal coordination compounds (MCCs) in inorganic biochemistry, but is mainly concerned with presenting:

i) some new characteristics of ionic (*i.e.* positively-charged) MCCs with reference to their chemical and biological implications.

ii) comparative data on the interaction behaviour of diverse types of (poly-) cationic biomolecules (to reveal some of their newer features) with urea, bile salts, EDTA (and congeners) dimethyl sulphoxide (DMSO), etc., (by serving as analytical tools) for characterizing the binary macroionic complexes.

Our interest in the use and in establishing the importance of ionic MCCs as models[9-15] in understanding their interaction behaviour in some of the biological/biochemical systems

stems out from a report on the precipitation of an acid [16] mucopolysaccharide by an ionic MCC, analogous to that of inorganic polyanions acting as models of anionic biopolymers [13–15]. Based on this concept, our initial studies suggested that inorganic polyanions could serve as simple and dependable models for understanding the interaction behaviour of anionic biopolymers with positively-charged dyes, drugs, antibiotics and scores of other biologically important materials. Hence in view of this fact, the review supported with relevant literature is presented under the following major headings:

i) Interaction of MCCs with ninhydrin
ii) Macroionic complexations:
   a) Interaction of cationic MCCs with anionic biomolecules/biopolymers.
   b) MCCs simulating the cationic biomolecules.
iii) Conclusions.

Table 1

Detection of metal coordination compounds (MCCs) with ninhydrin [17]

A. Positively-charged MCCs

No.	Compound	Colour and sensitivity (μg)			
		Spot test	μg	TLC	μg
1.	$[Co(en)_2Cl_2]Cl$	Deep brown red	0.5	Violet brown	8
2.	$[Co(en)_3]Cl_3$	Purple red	0.5	Pink violet	8
3.	$[Cr(en)_3]_2(SO_4)_3$	Deep violet	0.5	Violet	8
4.	$[Co(NH_3)_6]Cl_3$	Orange brown	1.0	Yellow	15
5.	$[Ni(en)_3]Cl_2$	Deep violet	0.2	Violet	5
6.	$[Co(NH_3)_5(H_2O)]Br_3$	Orange brown	1.0	Yellow pink	15
7.	$[Co_4(en)_6(OH)_6]Cl_6$	Dull red brown	0.5	Pink brown	8
8.	$[Cr_4(en)_6(OH)_6]Cl_6$	Violet brown	0.5	Violet pink	8
9.	$CoCl_2$	Pink	10.0	Pink yellow	40
10.	$NiCl_2$	Yellow	10.0	Yellow	40
11.	$Cr_2(SO_4)_3 \cdot K_2SO_4$	Green grey	15.0	Green grey	60

B. Detection of neutral MCCs with ninhydrin

1.	$[Pt(NH_3)_2]Cl_2$	Yellow green	1.0
2.	$[Li(Sal)_2]$	Brown violet	0.5
3.	$[Cd(Sal)_2]$	Brown pink	0.5
4.	$[Zn(Sal)_2]$	Pink yellow	0.5

en: ethylenediamine
sal: salicylic acid.

2.1. *Interaction of MCCs with ninhydrin*

Table I depicts the development of a simple and sensitive ninhydrin colour reaction[17] with MCCs to assess their homogeneity, importance/involvement of metal cations for macroionic binary complexation/interactions[18,19].

It is interesting to note that although the incorporation of metal ions in the ninhydrin reagent has been in practice[20] for a long time, hardly any attempt seems to have been made to understand the role of metal cations in this reaction[21]. Hence in our recent studies we have extended the use of ninhydrin to many MCCs (Table I), thereby confirming the importance of metal cations in the ninhydrin colour reaction which indirectly implied its analogy to the interaction of ninhydrin with amino acids and other reactive nitrogenous compounds[20,21] Further, it has been shown since the early fifties that ninhydrin gives colour reaction with some of the non-nitrogenous compounds[22–24] as well. In this respect, our investigations (unpublished data) have offered some new information, *viz.*, a non-nitrogenous compound like salicylic acid became reactive with ninhydrin, when present as a constituent ligand in an MCC. Incidentally, mention may be made of the development of a very simple and sensitive chromatographic method for the detection of different metal cations with specific colour shades and sensitivity ranges during our studies on ninhydrin interactions with MCCs (Table I).

These observation offer a new area of applied and fundamental importance for research in inorganic and analytical biochemistry.

2.2. *Macroionic complexes*

Macroionic interactions represent complexations between oppositely-charged species, one or both of which could be macromolecular in nature. Though generally electrostatic linkages represent the primary binding force in their formations, the non-ionic forces could also come into play in the entity of such complexes, and quite prominently[25–33] at times. The electrostatic, as compared with other types of interactions, are considered to be non-specific in character.

Bile salts and some ionic detergents have been extensively employed for the solubilization of enzyme systems and biological structures[34–38]. Likewise, EDTA has also been known to affect the cellular structures[39–41]. But no systematic study seems to have been made in understanding the influence of these reagents on macroionic complexes at molecular level. Hence, we believe ours is the first attempt in this direction.

A comparative study of the behaviour of macroionic complexes formed between a variety of oppositely-charged biologically important substances, including MCCs as models, reveals a new non-chelating[18,19] property of EDTA[42] (and its congeners) of dissociating binary complexes into their interacting components by rupturing the ionic linkages involved in their complexation. Nonetheless, EDTA has its own limitations because of its inability to dissociate some of the complexes whenever the primary and secondary binding forces taken together are probably too strong for this reagent to destroy them. The unusual property of EDTA[18] has been made use of by us for reversing the polyanionic inhibition of RNase[43]

(which does not depend upon metal cations for its activity) and by two groups of investigators in other contexts[44,45]

Further, in a search for alternative decomplexers it was considered appropriate to study the effect of deoxycholate (DEOC), dodecylsulphate[18,19] (SDS) and other bile salts on binary macroionic complexes including those involving MCCs. Their interaction with several hundred binary macroionic complexes attributed them the complex-breaking ability, however, with their own profile[18,19] of competence and limitations. To exclude the possibility of any incorrect interpretation, experiments were designed i) to rule out the role[42] of chelating action in the case of chelating agent(s) and ii) to distinguish the detergent action from the complex-breaking action in the case of SDS, DEOC and congeners. Besides,

i) to know about the ability of cationic MCCs, like the basic biomolecules to form complexes with various anionic biopolymers,
ii) to understand the comparative behaviour of the binary macroionic complexes (involving MCCs and biomolecules) with the help of complex breaking agents and to characterize the latter as well in the process,
iii) to establish for the specificity and non-specificity of complex formation as a result of the interaction of the MCCs with cationic biomolecules or bio-analogures. For instance, ionic interactions are generally believed to be non-specific in character, whereas in the living systems polyionic substances are established[25-33] to be a very specific and hence,
iv) to see if the MCCs could simulate the biomolecular substances and processes in some of their properties.

Thus, like inorganic salts and bile salts, urea and dimethylsulphoxide[46] (DMSO) could also dissociate some of the well-defined macroionic complexes including metachromasia (for definition see section 2.2.2) though[18,19] the two reagents were relatively weaker than the other decomplexers. By expressing this property towards macroionic complexes, urea and DMSO[46] are undoubtedly breaking up electrostatic linkages[18,19]. This implies a new characteristic attributable to them. However, these reagents are qualitatively and quantitatively less efficient than EDTA and bile salts, each displaying its own peculiarity of competence and limitations[18,19]

Further studies have revealed that bile salts/anionic detergents, can themselves form binary complexes with positively-charged MCCs and a variety of other basic biomolecules[19], thereby greatly adding the scope of this type of reaction to anionic detergents[36]. These binary complexes were observed to get dissociated in an excess of the interacting anionic detergent as reported in the case of (poly-) anionic interactants[10,25] Hence in the light of our experimental data[19] the precipitating action of bile salts by positively-charged substances[36] (including MCCs) may be considered to be a non-specific interaction[19] characteristic of the anionic detergent(s). We believe, therefore, that these newly observed features of bile salts, anionic detergents and chelators could have many biochemical and biomedical ramifications for a better understanding of:

a) the side effects of EDTA and similar chelators[42] when employed as therapeutic agents for the detoxification of heavy and radioactive metals[42,47] and

b) the deleterious action(s)[36,48] of bile salts/acids in bile acid atresia and other pathological conditions associated with their elevated levels in the living systems[49–51]; exploitation of the concept in biological systems is under experimentation.

2.2.2. Interaction of MCCs with anionic dyes and bio-molecules

Some of the positively - and negatively-charged dyes can interact with oppositely-charged substances (called chromotropes) as a result, suffering shifts in their λ maxs to lower wavelengths. This phenomenon, a reversible physico-chemical process not dependent either upon pH change or chemical reaction, is termed as metachromasia[52–57]. In this phenomenon the anionic (bio-) polymers act as chromotropes for the cationic dyes, while some of the (poly-) cationic biomolecules serve as chromotropes for the anionic dyes. By analogy to the second type of chromotropes, the MCCs could be shown[58–60] to induce strong metachromasia in the appropriate anionic dyes stoichiometrically[12], and serve as analytical tool(s) for localizing metachromatic components in the dye after its paper chromatographic resolution[58]. This technique has been exploited by other investigators[61–63] in the study of dyes. By simulating the (poly-) cationic biomolecules, the MCCs have revealed new properties in the case of urea, DMSO, EDTA (and congeners), bile salts (and anionic detergents)[18,19,33,34,36,42,46,48] These observations will open up many new areas of research.

The above studies suggest the interactability of the MCCs with various anionic (bio-) polymers. In fact, the available literature on complex formation between nucleic acids, acid polysaccharides, inorganic polyphosphates on the one hand, and proteins, protamine and other biomolecular substances on the other, point to the possibility of interaction of positively-charged MCCs with various polyanions[16,25–34,56]. The formation of a complex and its subsequent solubilization with a complex-breaking agent[19,30,43], KCl, as well as EDTA or bile salts for determining its titre value-an index of the mutual binding affinity and intensity of complexation of the two interactants forming a complex were carried out in the light of the methods of Scott[30], Moskowitz[64], Barber and Noble[65] with requisite modifications[10,18,19]. Accordingly, a study of these polyanions with MCCs showed that the intensity of complexation of the acid polysaccharides increased in the order; hyaluronate, ChS, heparin and dextran sulphate. This profile was exactly similar to that reported earlier by Scott[30] for these anionic polyelectrolytes when complexing with another basic compound, cetyltrimethyl ammonium halide. Commercial ChS (NBC, USA) was observed to be weaker in its interaction behaviour than the well-defined polysaccharides, ChS —A, —B, —C, —D. The various MCCs also differed among themselves in their interaction with the acid polysaccharides; Compound 8 was most potent, which was followed by compound 4 or 7 (Table I). The sterioisomeric character of an MCC also influenced its interaction behaviour. Thus, compound 1 (Table I) in its *cis*-form could interact with ChS, heparin and DS, while its *trans*-form did not show any visible interaction or only a very weak interaction with these polyanions; A more or less similar marked qualitative difference was also observed with N-DNA[66–68] and in other experimental systems.

Inorganic isopolyacids, condensed phosphates, in which the monomer is the inorganic phosphate, have the unique distinction of being synthetic as well as of biological origin[56,69–71] As occurring in biological systems and being associated with certain biochemical roles

including that of 'phosphagen', they have been categorised as 'soluble' and 'insoluble' polyphosphates depending upon the ease of their extraction into cold or hot trichloroacetic acid respectively[56,69,70]. Yoshida[70] had suggested that the two categories of polyphosphates might be resulting from the difference in their binding behaviour with proteins or other compounds of the concerned biological system. This was put to test by us by making use of the ability of inorganic polyphosphates to form complexes with MCCs and other (poly-) cationic compounds[10,18,19]. Accordingly, a profile of the binding behaviour of these polyanions into two categories, namely, the lower and the higher ones, which correspond with the 'soluble' and 'insoluble' forms of polyphosphates as propounded by Yoshida[70] was observed (and unpublished data with MCCs). This was the first experimental proof[10] of Yoshida's view about inorganic polyphosphates of biological origin. Several other studies employing difference parameters also demonstrated a clear demarcation between lower and higher polyphosphates[71–74].

As in the case of acid polysaccharides, the *trans*-form of the MCC, compound 1 (Table I) unlike its *cis*-form, was inert or poorly interacting/reactive with the inorganic polyphosphates[74]. The Cr coordination compound (compound 8, Table I) was found to be the strongest of all the studied MCCs in its complex formation behaviour with these polyanions. Between the two categories of the lower phosphates, the tri-and tetra-mers, the cyclic forms (metaphosphates) were poorly reactive or non-reactive with the MCCs and other polycationic compounds as against the corresponding open chain polyphosphates[71,74].

Lastly, it is worth mentioning that the MCCs displayed some of the following novel features in their interaction behaviour with the third category of the anionic biopolymers, the nucleic acids[66,74,75]

Clot formation: Only the double-stranded (Native, N—) DNA formed clot with the MCCs while all other tested polyanions, acid (muco-) polysaccharides, inorganic polyphosphates, RNA, synthetic polyanions such as polyanethol sulphonate, polymethylene salicylate, polyestradiol phosphate, polyphloretin phosphate, etc., gave precipitate with the MCCs[66].

Cis-vs *trans-form of an MCC:* Only the *cis*-form of compound 1 (Table I), $[Co(en)_2Cl_2]^{1+}$ interacted with N-DNA as a clot, while the *trans*-form was non-reactive or poorly reactive with this polyanion[66,74]. In this respect, the interactive MCCs were behaving like (poly-) cationic substances. This study describing a marked difference between the *cis*-and *trans*-form of an MCC[8,76–78] predates any such report in the literature; it was only some years later that *cis*-platinum compounds as against the corresponding *trans*/ones were shown to interact with DNA and exhibit antitumor activity[76–78]

DNA, RNA protection: From the noted novel features, it was, therefore, surmised that like the (poly-) cationic substances[79–91], the MCCs might also exhibit protection of N-DNA against temperature denaturation. This was in fact found to be so with the help of a new technique called heterogeneous phase system (HPS) which, as mentioned above, involves the expression of unique characteristic property of N-DNA of clot formation with many (poly-) cationic compounds including the MCCs. This brings N-DNA into a compact and

highly concentrated state[66,74,75]. In this form, this biopolymer, simulating its natural status as complexes, was successfully employed for assessing its protection[92-94]. This is quite unlike the conventional method of Tm (temperature of melting of DNA) determination, wherein DNA is employed in its free form and in very low concentrations[79-83], which do not commensurate with the natural status of the biopolymers[92-94].

Another new feature of the approach is the protection of DNA even by those MCCs which contained cations of Ag, Hg and Pb as their constituent components[95]. These cations are known to destabilize the double-stranded structure of the biopolymer[96] probably by involving the bases in their interaction with it, which leads to the weakening of the inter-strand hydrogen bonds and hence destabilization of the double-stranded structure[79-81,83]. The stabilizing effect of the MCCs of Ag, Hg, Pb (cations), may be explained by their electrostatic interaction with N-DNA probably not involving any interaction(s) with the DNA bases, which has an adverse effect on its stability[96]. The above results were further confirmed by subsequent reports on the protective action[97,98] of transition metal MCCs towards DNA. Lastly, a word about lanthanides which also exhibit protective action against N-DNA by more or less a similar mechanism of clot formation as that of transition metal MCCs. An extrapolation of these and similar studies afforded some clue towards[74,75] DNA protection against other harmful agents[99-101] like H_2O_2, sonication, protonation, free radicals, etc., on which it appears very meagre information is available in literature[79-91]

Table II
Protection of RNA complexed with (poly-) cations against the action of RNase

(Poly-) cations	Total time of hydrolysis (h)	% Protection
Protamine	26	87.0
Lysozyme	26	81.0
Histone	26	79.0
Haemoglobin	26	82.0
$[Co_4\ (en)_6\ (OH)_6]^{6+}$	30	87.5
$[Co\ (NH_3)_6]^{3+}$	30	76.5
$[Co\ (NH_3)_5\ (NO_2)]^{2+}$	30	80.0
Viomycin	26	88.0
Neomycin	26	83.0
Streptomycin	26	81.0
Kanamycin	36	83.0
Bovine serum albumin	36	86.0
Polymixin	36	84.0
Tofranil (Imipramine)	36	88.5
Samarium chloride	48	84.0
Propamidine	48	93.0
Phenylbutazone	48	76.5

As a natural sequel to the above[74,75,95], a comparative study of the insoluble complexes of RNA with MCCs and other polycationic compounds clearly demonstrates the protectability of this biopolymer against prolonged and repeated action of large amount of RNase[102] (and unpublished data). The protective action of compounds 4, 7 and 8 (Table I) is practically equal to that of well-known protectors such as protamine, polymines, etc. (Table II)[102].

The optimum inhibitory effect of various (poly-) cationic compounds in the homogeneous phase towards RNase is always less than that shown in the HPS system when RNA is present as its insoluble complex with a positively-charged compound, whereas in the soluble form the thermodynamic freedom of the substrate and hence its susceptibility to the enzyme action is far greater such that its enzyme susceptible sites get masked to the maximum possible extent by a complex forming compound[80,81,83-87,91,102-105].

This suggests that:

i) the operation/existence of subtle difference in the complex formation process between the nucleic acid and a polycationic compound.
ii) the manner of (degradative) action of the different harmful agents (like H_2O_2, sonication and nucleases) on RNA/DNA may be inferred to be not similar in nature.
iii) the new method is both quite sensitive and reproducible so as to be able to register the above subtle differences in the behaviour of nucleic acids.

Thus, because of their altered entities[27,31-33,43,52,53,81,83,91] coupled with an increase in their stabilities ('life') the 'native' biomolecules may behave as 'foreign' ones invoking the serious risk of 'autoimmune' reactions, implying a new role for/effect of xenobiotics[102-110].

2.2.3. MMCs simulating the cationic biomolecules

In the light of the data presented in the previous section in order to assess the MCCs simulating cationic biomolecules, two experimental set-ups have been chosen:

i) *in vitro* antiheparin ability (since preliminary results had shown the MCCs to be interacting with heparin), and
ii) interactability with microbial cells and cellular organelles (because of the overall anionic nature of their outer surfaces)[112,113].

The compound 8, $[Cr_4\ (en)_6\ (OH_6)]^{6+}$ exhibited antiheparin potency[114] equal to that of toluidine blue but one fourth of that of protamine. This approach was extended to other types of positively-charged MCCs, various cationic drugs of cationic metabolites for their antiheparin potential, and thereby motivating this investigation for a better understanding of the blood clotting system in relation to heparin[114].

Now as regards the effect of MCCs on cells and cellular organelles, the positively-charged MCCs behaved like protamine and polymines by bringing about agglutination of the living cells and cellular organelles[11]. It was further shown that the above cationic biomolecules and MCCs could also prevent the efflux of materials absorbing at 260 and 280 nm from the rat liver mitochondria in the hypotonic milieu[11]. The MCCs could also serve as a sensitive tool for differentiating *E. coli* from *Vibrio* cholerae cells[11] since the latter were found to be much

less susceptible to the agglutinating action of MCCs. An extention of this approach to other types of cells including the normal and abnormal mammalian cells, therefore, appears to shed more light on this topic.

The results of the above investigations on the positively-charged MCCs *vis-a-vis* widely varied experimental systems have clearly demonstrated their ability to simulate the cationic biomolecules in regard to all of their tested characteristics. It may also be inferred from the above that the results of the *in vitro* studies may be applied to *in vivo* systems with a good deal of confidence and reliability for a better understanding of biological/biochemical processes at molecular level.

3. Conclusion

The present series of study[9-15,17-19,43,58-60,66,72-75,95,102,108,114] based upon nonconformistic but simple approaches centering around MCCs as applied to modern biochemical and biological problems has attributed several new properties not only to MCCs, but also to bile salts (and other anionic detergents), EDTA (and congeners), urea and dimethylsulphoxide, revealing new features of inorganic polyphosphates, and their implications in cytochemistry. This approach has been shown to help in evolving new methods for determining the stoichiometry of metachromasia, in the differentiation of native DNA from the denatured one, leading to a new technique for the study of protection of RNA/DNA aganist a variety of harmful agents, in revealing the stereospecific macroionic interactability of *cis* form of an MCC as against the *trans* one, in finding a new use for ninhydrin, in differentiating one type of bacterial cells from another, etc.

It was considered to offer new possibilities and concepts in biomedicine, especially in the modulation of drug action by bile salts, urea, DMSO, etc., in relation to pathological conditions when elevated levels of these substances prevail in the living system. These studies, establishing metal coordination compounds as model avenues for understanding and modulating the effects and side effects of chemotherpeutic agents and xenobiotics will facilitate in devising new approaches for counteracting their undesirable effects.

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