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Biological metal ligand systems

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Abstract - There are some discrepancies between the behaviours of the metal-ligand biosystems and the lifeless systems. The participation of macromolecular ligands might be considered as the most important origin of these discrepancies. The amphivalency, flexibility, adaptation to coordination configurations and the availability of the metal-binding sites determine the characteristics of the metal(ligand) exchange reactions. The metal-cell interactions have been demonstrated as another feature of metal-ligand systems in living organisms. The cells, as multiple-metal-multiple-ligand systems, response to metal ion or complex holistically. The compartmentalization of the ligands results in the localization and orientation of the reactions involved and thus shows various reaction patterns. Another factor emerging in biosystem and affecting the metal-ligand reactions is the biological medium. A special medium effect has been studied for the bile system, in which some surfactants and polyelectrolytes present and influence the ionic reactions occurring in bile.

INTRODUCTION

The increasing understanding about the contributions of biometals to life process has provided a great challenge to chemistry. This situation has prompted some coordination chemists devoting to the studies of the characteristics of biological metal-ligand systems (hereafter denoted as M-L system). As the pioneer works, Perrin's (ref. 1) and Williams' (ref. 2) models, simulating the M-L systems in plasma and gastrointestinal fluids, offered us an approach to gain an overall insight to the speciation of metals in human body. Their working premises may be generalized as follows:

1. The speciation of metals and ligands are interdependent through the multiple equilibrium of numerous protonation and coordination reactions.

2. By solving the simultaneous equations describing the equilibrium states of these reactions, the states might be predicted.

Obviously, these treatments are valid only for the systems fulfilling the following restrictions:

1. The equilibrium state has been established for all the reactions involved.

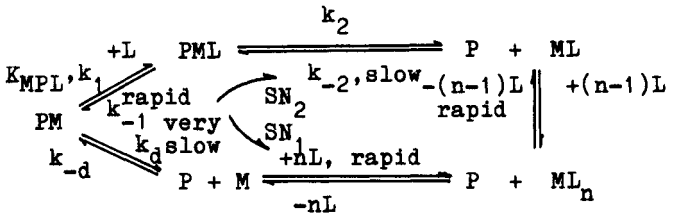
2. All the reactions follow the general laws of the solution chemistry of coordination compounds.

The first point is usually complicated in life systems, because these systems are opened in nature and thus they can never attain equilibrium, except for the very rapid reactions with long retention time. However, this problem might be solved by means of static state approach. Such treatments emphasize the kinetic aspects of the reactions and require all the kinetic parameters determined or estimated. Even though the first problem might be solved, we cannot develop a successful model without the consideration of the features of the biological M-L system concerned. In the present paper, some aspects of the characteristics of the M-L systems of living organisms will be discussed.

METAL EXCHANGE REACTIONS INVOLVING MACROMOLECULAR LIGANDS

Among the reactions which occur in M-L systems, the metal exchange reaction is probably the most prevailing and important type of reaction. In pure coordination chemistry, it is still the main subject of mechanism studies, from which some important conclusions have been drawn. Nevertheless, it is still vague whether these conclusions hold exactly in biosystems as well.
The involvement of macromolecular ligands, P, complicates these reactions and leads to some behaviours worthy to be rethought and restudied.

Supposing the mechanisms of various metal exchange reactions might be generalized to the following scheme, various modes of reactions might be discussed on the basis of different rate constants k and stability constant K.



We have suggested a criterion system to discriminate the different reaction modes on the basis of spectrophotometric studies of P-M-L systems (Table 1).

TABLE 1. Criteria of mechanisms of metal exchange between macromolecular ligand P and low molecular ligand L

Mechanism	Conditions		Criteria*
Addition	PML rapid formation slow dissociation	PML not negligible	$1/C = 2.303/k_2K_{PML}[L]_o + 2.303/k_2$ $1/C \sim 1/[L]_o$ linear
		PML negligible	$C = k_2K_{PML}[L]_o/2.303$ $C \sim [L]_o$ linear
	PML formation and dissociation overlap	$k_2 \ll k_1, k_{-1}$	$B_4 = -(k_{-1} + k_1[L]_o)$ $B_4 \sim [L]_o$ linear
		$k_2, k_1 \gg k_{-1}$	$B_2 = -k_1[L]_o$ $B_2 \sim [L]_o$ linear
Dissociation			$C = k_d/2.303$ C independent with $[L]_o$

* C, B_2 and B_4 are obtained from spectrophotometric data

These criteria have been used to elucidate the mechanism of the mobilization of human serum albumin(HSA)-bound copper and bovine serum albumin(BSA)-bound nickel with low molecular ligands L. The results show that the structural characteristics of macroligands and their complexes play important role in determining the mechanism of metal exchange reactions.

1. Among the geometric configurations of metal binding sites of the macromolecular complexes, the square planar configuration will be much more favoured for ternary complex formation than the tetrahedral or octahedral configurations, and thus the addition mechanism will be preferential for these cases. For example, the square-planar coordinated Cu(II) in HSA is mobilized through a ternary complex with various chelators, while in the case of octahedral Ni(II)in BSA, no ternary complex has been found.

2. If the macromolecular ligand is rigid and the metal binding sites are buried in the cavity, the structures of low molecular ligands should match the cavity and site for mutual binding. If the macroligand is flexible enough to envelope the attacking ligand, the requirements will be less restricted.

3. The addition mechanisms are preferable for the linear macroligands, such as HSA, for the sake of their open conformations.

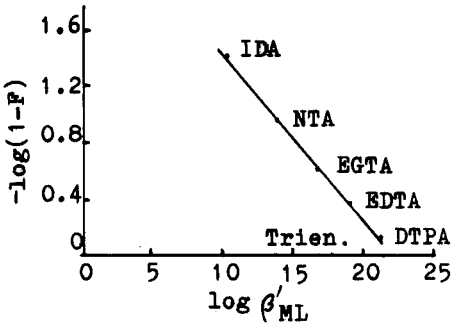


Fig.1. Relationship between log(1-F) and logβ' for mobilization of HSA bound copper(II)

If we neglect the characteristic of individual ligands, the relative mobilizing power of different complexing agents toward a certain metal bound to a certain macroligand is commonly expected to vary in parallel with the metal binding abilities (conditional stability constants β' as a measure of it). With the consideration of the involvement of macroligands, we have suggested a factor F to evaluate the competitive power of ligands L in the ternary system (M-P-L). F values may be determined experimentally as follows:

$$F = \frac{\text{Amount of metal bound to P in presence of L}}{\text{Amount of metal bound to P in absence of L}}$$

Theoretically, F relates with β' by the following equation:

$$(1/F)-1 = \beta'_{ML}[L]/\beta'_{MP}[P]$$

The linear relation of log(1-F) and log β'_{ML} is shown as Fig. 1.

The deviation from the linear relation might be considered as the indication of some special interrelations between L and P.

Another noteworthy feature is the nonreversed relation of the following pair of reactions:

$$ML + P \rightarrow MP + L \qquad (1)$$

$$MP + L \rightarrow ML + P \qquad (2)$$

For mobilization of HSA bound copper with different chelators (reaction 2), the sequence of the observed rate constants is

EDTA > DTPA > EGTA > NTA > IDA

while for the transport copper to HSA (reaction 2), the sequence is found to be just reversed:

IDA > NTA > EGTA > DTPA > EDTA

By means of equilibrium chromatographic studies, the equilibrium states of HSA-Cu + L and CuL + HSA were compared. The results indicate that the amount of HSA bound copper is higher, if the reaction starts from HSA-Cu + L.

All the above mentioned observations might be attributed to the following characteristics of macromolecular ligands:

1. Amphivalency: There are a number of potential binding sites with different affinity and different modes in macroligands.

2. Availability of binding sites: It is determined by the structure, but influenced by pH, ion strength, etc. of the medium.

3. Flexibility: Metal or ligand binding induces conformational change, which may be transmmitted by allosteric effect and alters the affinity to metal or ligand.

INTERACTIONS BETWEEN METAL COMPLEX AND CELL

For the living cells, one of the most important processes is the interactions between the cells and the exogeneous substances, which will be the metal complexes in our concern. In the course of the interaction, every cell responses as a system comprising a number of target molecules. Each of them is confined

to a destined compartment, leading to various reaction patterns. As the complex attacking the cell, the latter manifests as a M-L system, but differs from that in bulk solution. In a solution system, the metal ions and ligands are in random motion and interact stochastically. The ligands would strive for the binding with metal ions. Naturally, all the reactions follow the kinetic and thermodynamic principles. The diversity of different ligands results in selective coordination and produces the distribution pattern for every species. In a cell system, due to the compartmentalization of ligands, the reactions are localized and directional in space scale and arranged as a sequence of events in time scale.

Cisplatin and its analogs, as a group of anticancer complexes, have been studied extensively for their pharmacological mechanism. Its anticancer action is generally recognized as the result of DNA damages. Our recent observations indicate strongly that their activity is the holistic response of the whole cell system. In other words, it is the overall results of all the reactions between cisplatin and target molecules.

First of all, by means of FRAP technique, we have found that the lateral diffusion of membranic phospholipid molecules, measured in terms of $\bar{D}$, increases and the recovery rate ($\bar{R}$) is retarded after incubation of the ascitic liver cancer cells with a platinum complex, dichlorodimethyldopamidoplatinum(II), MDP, as shown in Table 2.

The studies on cisplatin-liposome system revealed that the platinum complex binds to phospholipid after the Cl^- ions are displaced by water molecules.

We have identified DNA molecules in the membrane of ascitic liver cancer and S180 cells. With FRAP again, we have found that $\bar{D}$ values are not sensitive to platinum complex, but the recovery slows down. Nearly one third of DNA molecules are not able to approach their original position. (Table 3)

It is very interesting that the platinum complex renders the cytoskeleton networks disappear to some extent under fluorescence microscope, with F-actin stained by fluorescence probe. This effect might be attributed to the direct action of platinum complex on F-actin, but the indirect action mediated by Ca^{2+} ions cannot be excluded. In the latter postulation, the sequence of events is thought to be:

Pt binding → induced free radical generation → oxidative damages to cell membrane → Ca^{2+} influx → Ca^{2+} induced conformational change of F-actin

A similar sequence has been suggested for the interpretation of blebbing of membrane of hepatocytes damaged by free radicals (ref.4).

Since nuclear DNA is the innermost target, we attempt to clarify whether the platinum complex molecules approach DNA after they have damaged the skeleton proteins throughout the way. But, as we have studied with ascitic liver cancer and S180 cells, no parallel effect or any cause-effect relation has been found. These observations might be interpreted by assumption of the intracellular formation of cis$[Pt(NH_3)_2(H_2O)_2]^{2+}$.

In light of these evidences and the results of other relevant in vitro experiments, we postulated that the cell membrane will be the frontal target facing the attacking platinum complexes. The complex molecules tend to bind

TABLE 2. Action of MDP to membranic phospholipid molecules

Cell group	Time (min)	$\bar{D}$ ($\times 10^{-9} cm^2/s$)		$\bar{R}$ (%)	
		Control	MDP	Control	MDP
5x3	30	4.51±0.8	5.68 ± 1.9	65 ± 0.21	55 ± 0.07
5x1	60	2.16±0.9	2.16 ± 0.5	61 ± 0.11	37 ± 0.11
5x4	60	3.11±1.4	3.71 ± 1.5	55 ± 0.16	44 ± 0.18

TABLE 3. Action of MDP to membrane associated DNA molecules

Cell group	Time (min)	$\bar{D}$ ($\times 10^{-9} cm^2/s$)		$\bar{R}$ (%)	
		Control	MDP	Control	MDP
5x6	30	6.59±3.0	6.02 ± 1.79	82 ± 0.34	58 ± 0.11
5x5	60	4.26 ± 1.14	4.11 ± 1.44	100 ± 0.19	68 ± 0.23

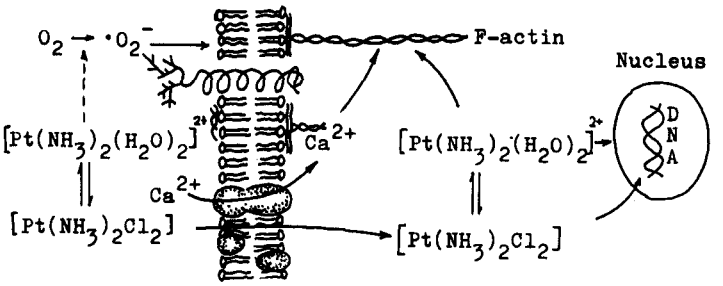


Fig. 2. Response of cells to cisplatin

to different ligands. According to the kinetic and thermodynamic behaviours of different ligands, some metals might be released and the damages repaired. The metal complex molecules will be transferred among various ligands in such a way until they bind to the thermodynamic stable sites. As the final stages, their effects are transmmitted to intracellular targets and cause intracellular damages.

Thus the holistic picture of the cell response may be illustrated as Fig. 2. It is believed that the inhibition or death of cells are the collective response of various target molecules instead of the damage to a single target.

MEDIUM EFFECTS ON IONIC REACTIONS

Another indispensible consideration is the effects manifested by the specific medium of the M-L system considered. Conventionally, one predicts the states of such a system by solving a group of simultaneous equations of some thermodynamic and kinetic parameters. They are all determined in simple solutions, with specified pH, ion strength and temperature. The values thus obtained are only applicable for the systems under the same conditions. Since we are applying this approach to a certain biological system, such as the human bile system, the fidelity of the results would be questioned, even though the parameters used have been determined in a solution, whose pH, temperature and ion strength are all consistent with the physiological conditions. There are some additional factors should be considered. For instance, the bile is a quasisolution system containing metal ions, anions of some organic acids, cholesterol, bilirubin, bile salts and mucoproteins. The sparingly soluble bilirubin and its calcium salt and cholesterol are solubilized by the special micellar medium composed mainly from bile salts. The mucoprotein, as polyelectrolyte, may transform into hydrogel and influences the medium. In consequence, this is a M-L system with a micelle + hydrogel background. Such a background exerts profound influences on all the ionic reactions in bile.

The reaction between Ca^{2+} and bilirubin, leading to the precipitation and agglomeration of calcium bilirubinate, is a rather simple reaction:

$$Ca^{2+} + B^{2-} \rightleftharpoons CaB_{(s)} \qquad B^{2-}: \text{ dibasic anion of bilirubin}$$

It is complicated by the bile background. The medium effects keep the calcium bilirubinate in solution, or in finely dispersed form. This is essential to keep the bile to be nonlithogenic.

Even a very low concentration of bile salts (less than CMC) tends to increase the solubility product of calcium bilirubinate, retard the rate of precipitation and inhibit the agglomeration of calcium bilirubinate. These effects may be further enhanced by higher concentration of the bile salts. As one of the typical results, the conditional solubility products (pK'_{sp}) and rate constants were given in Table 4 as the functions of concentration of sodium taurocholate. The influence on the distribution of particle size was clearly shown in Table 5. (ref. 5.)

TABLE 4. Conditional solubility products (pK'_{sp}) and rate constants (k) of calcium bilirubinate as function of concentration of sodium taurocholate (C) 37±1°C

C (mM)	0	5.16	10.05	15.25	20.05
pK'_{sp}	15.73	15.01	14.48	13.72	12.54
k (s^{-1})	6.3×10^{-1}	5.71×10^{-2}	1.43×10^{-2}	3.97×10^{-3}	5.61×10^{-4}

TABLE 5. Particle size distribution of calcium bilirubinate in presence of sodium taurocholate (TC)

TC (mM)	Characteristic parameter	shape coefficient	average diameter (μm)	variance
0	1.51	1.62	1.553	0.996
10	0.765	1.09	1.141	0.537
15	0.386	0.74	0.868	0.256

The solubility, rate of crystal formation and degree of agglomeration are the essential factors determining the probability of stone formation in bile, urine etc. The medium effect of bile salts inhibit gallstone formation from these aspects. The anions of bile acids were shown to bind with calcium ions and bilirubin, either in molecular level or in micellar level, and convert them into a special state--micelle-binding state.

All the attempts to dissolve or disintegrate the calcium containing gallstone with a calcium chelator, such as EDTA, are not successful. We have studied the dissolution process of the following reactions (ref. 6.)

$$CaB_{(s)} + L \rightleftharpoons CaL + B^{2-}$$

The results show that the dissolution of calcium is not synchronized with that of bilirubin and a network of bilirubin remained as an insoluble residue. In presence of cholate, the dissolution of both calcium and bilirubin are enhanced. The synergic effect of bile salts and chelators might not be explained in terms of solubilization of bilirubin solely. The relevant equilibrium constants (solubility probuct, dissociation constants and stability constants) have been determined in presence of various bile salts. Thus the medium effect of bile acids toward ionic equilibria may play important role in the formation and dissolution of gallstone.

When two or more surfactants coexist in a solution, their mutual action may be synergic or antagonic depending on their structure and property. We have studied a system containing Ca^{2+}, bilirubin, cholate and an additional surfactant, 1-hydroxyethanediphosphonate (HEDP). HEDP anions are able to bind with the calcium ions on the surface of a solid calcium salt particle, with the formation of a surface polymeric complex. The masking of the active centers of crystal surface will inhibit the growth of crystal or, in a certain case, influence the agglomeration of particles. In addition, the surface complexation of HEDP enables it joins with two or more solid particles and thus enhances the agglomeration. From the experimental studies of the particle size distribution of calcium bilirubinate, we have found that, in presence of HEDP, the particle size becomes smaller somewhat, but the coexistence of cholate promote the inhibition significantly. A number of reasons may be raised to interpret the synergic effect.

In addition, we are also interested in the mutual influence between a surfactant and a polyelectrolyte, as we have encountered in the studies of bile system. The mucoprotein has been postulated as the essential factor for gallstone formation. We have determined the rate of precipitation and degree of agglomeration of calcium bilirubinate in presence of chondroitin sulphate. The precipitation is accelerated and the particle size increases. In a calcium-bilirubin system, inhibited with bile salt, the addition of chondroitin sulphate counteracts the action of bile salt.

CONCLUSION

The three problems discussed above are by no means the most important and dominant aspects in biological M-L systems. My aim is showing a picture of several features of these systems and searching an approach to solve these problems. All of the processes in biological systems are of course consistent with the general laws of Nature, including the thermodynamic and kinetic principles. However, to interpret any phenomenon in biological M-L systems, the introduction of some additional conditions are necessary.

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