CHEMISTRY & CHEMICAL TECHNOLOGY

Vol. 5, No. 1, 2011 Chemistry

Liya Khmars'ka[1]*, Kostyantyn Kylyvnyk*[2] *and Olexandr Shtemenko*[1]

INFLUENCE OF Ni(II), Cu(II) AND Zn(II) COMPLEX FORMATION ON INTERACTION WITH MODEL CELLULAR MEMBRANES

[1]*Ukrainian State University of Chemical Technology, 8 Gagarin Ave, 49005, Dnipropetrovsk, Ukraine*
[2] *NanoEner Inc., 33309, Fort Lauderdale, FL, USA*

Received: October 04, 2010 / Revised: October 25, 2010 / Accepted: December 12, 2010

Abstract: Influence of complex compounds of nickel(II), copper(II) and zinc(II) with nitrogen containing ligands on conductivity of bilayer lipid membranes (BLMs) was studied. The role of conditions (pH, metal and ligand concentration) on complex formation and interaction with BLMs was evaluated. Composition and form of complex ions in solution which maximally change conductivity have been calculated. Relationship between the complex form predominant in the solution and BLM conductivity indicated that complex ions influence mostly depended on surrounding ligands and did not depend on the nature of the central atom. Permeability coefficients for complex ions which maximally changed BLM conductivity were calculated.

Keywords: metal complex, nitrogen containing ligands, bilayer lipid membrane, BLM, conductivity, lipophility.

1. Introduction

Medicinal properties of metals and their compounds have been well-known since the times of Avicenna and Hippocrates. Discovery of antitumor activity of platinum complexes [1] anti-inflammatory activity of gold and copper complexes, and antianemic activity of iron and zinc complexes [2] increased scientific interest to metal complexes as potential medicinal agents [3]. Constant interest to such substances led to the necessity to synthesize a new class of coordination compounds with bioactive ligands as well as to systematize the search of new medicines on the basic of metal complexes [4]. At present great attention is devoted to the development of new medicines as well as investigation of the mechanism of their transport and their biological activity. Thus, metal complexes are a very attractive object of investigation with the aim of their further application in biology and medicine.

From the 60's of the last century, attempts to systematize the role of metal for living organisms have been undertaken. As a result several assumptions of coordination compounds influence on biosystems have been offered. Till now, establishing correlation between physical-chemical properties of metal complexes and their physiological activity remains the most important problem for the search and syntheses of new medicines based on complex compounds. Moreover, it is complicated by a variety of forms of complex particles presented in real-life physiological environment. The absence of systematic researches of membrane activity, analysis of composition, structure and form of complex ions that penetrate through cellular membrane impede further search of perspective bioactive metal complexes.

After intake any bioactive agent (including medicine) interacts immediately with cellular membranes and their components. As a result, functions of cells' membranes and cellular ferments change and "substance phenomenon" appears. Hence, determination of initial activity of medicine substance, *i.e.* mechanism of substance interaction with cellular lipid membranes, is of great importance for the creation of new drugs.

In this paper planar bilayer lipid membranes (BLMs) were formed from lipid mixture and used as a model of a cellular membrane. General fraction of bull's brain phospholipids contains lipids which are most commonly occurring in natural biological membranes. BLMs are an adequate model of lipid matrix and possess practically all of its physical and chemical properties [5].

An attempt was made to study the interaction of nickel(II), copper(II) and zinc(II) complexes with nitrogen-containing ligands and BLMs. Influence of complex's concentration, its saturation and pH on its capability to penetrate through BLM and to change its conductivity was determined. Investigation of metal ion penetration paths inside the cell is necessary for purposeful synthesis of complex compounds with directional biological action.

2. Experimental

2.1. Materials and Methods

In the present work bilayer lipid membranes (BLMs) were obtained from common fraction phospholipids of bull's brain white substance and then dissolved in *n*-octanol (20 mg/ml) by Folch method [6]. BLMs were formed on a small aperture in Teflon wall that divided two compartments with electrolyte solutions [7]. Mixture of KCl with 0.1N phosphate or 0.05M borate buffer was used as a background electrolyte. The pH of solution was constantly monitored by adding HCl or KOH.

Influence of the compounds on BLMs was estimated by the changes of such electrochemical characteristics as specific conductivity or penetration, membrane potential, capacity, *etc*. Changes in electrochemical properties were controlled by cyclical voltammetry [8, 9] within the range of ±100 mV. Resistance of BLM was determined by the slopes of current-voltage curves. All experiments were carried out at 291–293 K. The investigated complexes were injected into solution in microdoses and held for 15 min to attain equilibrium.

Metal(II) complexes with ethylenediamine and hydrazine were obtained directly in aqueous solution by mixing appropriate salts. Chlorides and nitrates of nickel, zinc, copper; 20% aqueous solution of ethylenediamine; and hydrazine chloride were used as starting salts. Complexes with α,α'-bipyridyl $[Cu(bpy)_3]CrO_4$ and $[Zn(bpy)_3]CrO_4$ (bpy – 2,2'-bipyridyl) were synthesized and kindly afforded by Prof. M. Cieslak-Golonka (Institute of Inorganic Chemistry and Metallurgy of Rare Elements, University of Technology, Poland) [10].

The formation of complex ions in the solution was confirmed by using spectrophotometric and potentiometric methods [11-15].

Speciation diagrams were used to evaluate influence of pH solution on the complex's forms and share. The diagrams were calculated and plotted by using software product CHEAQSPro [16] and HySS [17] and using stepwise metal-complex stability constants [18, 19].

3. Results and Discussion

As was determined earlier [20, 21], nickel(II), copper(II) and zinc(II) ions do not penetrate through membrane's lipid matrix either as aqueous complexes or as hydroxocomplexes. To determine influence of CrO_4^{2-} ions on BLM, electrochemical properties and additional experiments with K_2CrO_4 were carried out. It was discovered that chromate-ions did not change BLM conductivity, *i.e.* these ions do not penetrate through lipid bilayer.

When bipyridil complex is added to solution on one side of the BLM, current-voltage curve slope increases and zero-current point moves to negative potential area.

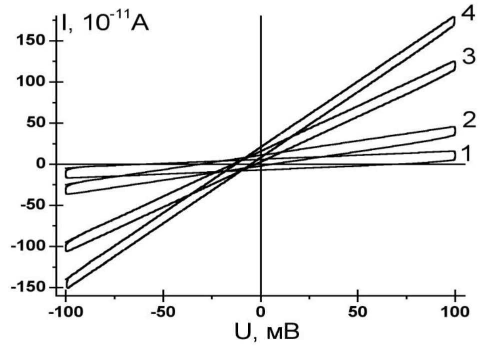

Fig. 1. Voltamperometry curves at addition of bipyridyl complexes: initial BLM (1); adding $2\cdot10^{-4}$, $6\cdot10^{-4}$, $8\cdot10^{-4}$ M $[Cu(bpy)_3]CrO_4$ (2, 3 and 4)

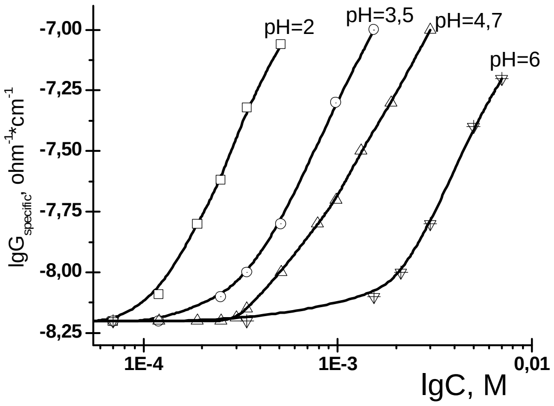

Fig. 2. Dependence of specific membrane conductivity on $[Cu(bpy)_3]CrO_4$ concentration at various pH

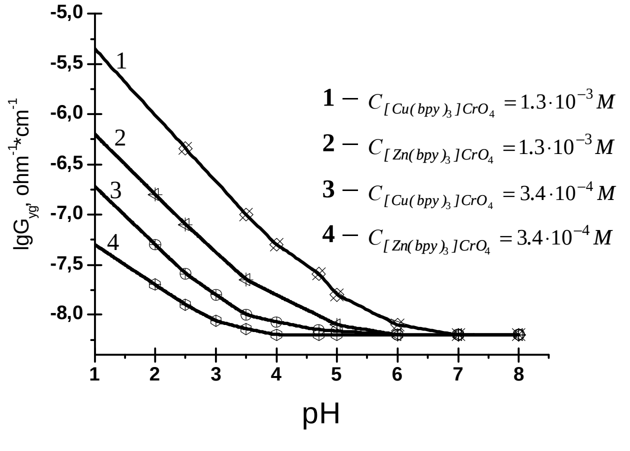

Fig. 3. Comparison of influence of zinc and copper bypiridyl complexes on specific BLM conductivity

It was established that membrane conductivity depends on the complex concentration and solution pH. Fig. 2 shows the dependence of specific membrane conductivity on concentration of $[Cu(bpy)_3]CrO_4$ for different pH.

According to Fig. 2, the influence of bipyridyl complexes on BLM conductivity decreases with the increasing of the solution pH and in alkaline medium it is practically absent.

The same experiments were performed for zinc complex and similar results were obtained. However, zinc complex influence on BLM conductivity is lower than that of copper complex at the same concentrations.

Speciation diagrams were calculated to determine different action of zinc and copper complexes on BLM. These diagrams describe form and share for each complex part depending on pH and concentration. The equilibrium constants, stability complex constants, formation of hydroxocomplexes, and solid phase were taken into account.

Comparative analysis of the speciation diagrams (Fig. 4) and influence of the complexes on BLM conductivity (Fig. 3) allowed us to conclude that coordination-unsaturated complex ion $[Me(bpy)]^{2+}$, which predominates at pH = 2, penetrates through the membrane and changes its conductivity. The difference in the behavior of Cu and Zn complexes can be explained by various shares of complex ions due to different stability constants (pK_1 = =8.2 and pK_1 = 5.2, respectively). If instead of the amount of the added complex the share of the complex ion is taken into account for the analysis of the influence on BLM properties, the difference disappears. Therefore it can be concluded that BLM conductivity changing does not depend on the nature of the central atom of the complex ion and only depends on the share of the complex ion.

Speciation diagrams for ethylenediamine complexes with nickel(II), copper(II) and zinc(II) were calculated and plotted based on the well-known constants [18]. Then, the complexes influence on BLM conductivity at various pH values was studied. It was determined that maximal changing of membrane conductivity observed when complex particle with three ethylenediamine molecules is predominant in the solution. It was also determined that in this case the nature of the central atom does not affect the capability to change BLM conductivity (Fig. 5).

Experiments were also performed with hydrazine complexes, and their influence on BLM was observed for larger quantity of ligands and narrower pH range. Analysis of "Me-hyd-OH" systems has shown that in experimental conditions coordination-saturated complexes, such $[Ni(hyd)_6]^{2+}$, are predominant. It was discovered that nickel complexes have more influence on BLM conductivity than copper and zinc ones. It can be explained by the presence of six hydrazine molecules in nickel complex and formation of more stable particle compared to Cu^{2+} and Zn^{2+}.

Further, comparative analysis was carried out for complex ions that maximally changed BLM conductivity (Fig. 6).

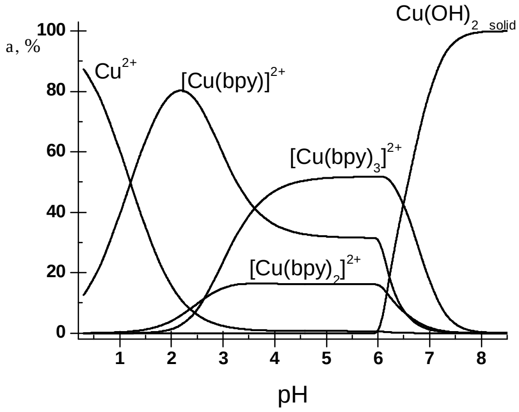

Fig. 4. Speciation diagram
for copper – α,α'-bipyridyl

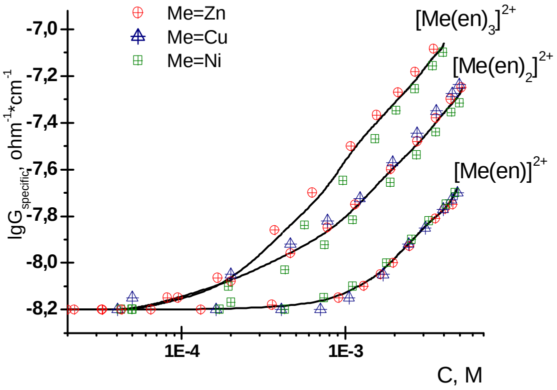

Fig. 5. Dependence of BLM conductivity
on concentration of various forms of complex ions in the
solutions with ethylenedianine (en)

Despite of differences in BLM conductivity changing, the mechanism of the complex interaction with lipid bilayer is identical in all cases. After addition of complex to the solution on one side of BLM, a slight shift of zero potential into the negative area was observed. This is the evidence of the adsorption of positively charged complex particles on the lipid bilayer. After that, the complex ions start penetrate through the lipid matrix and thus change BLM conductivity, which can be seen by the slope of the volt-ampere characteristic.

For subsequent analysis, interaction complex ions with BLM penetration coefficients (P) have been calculated. The calculations have been made for complex particles with ligand amount that maximally changed BLM conductivity. All measurements were carried out in the concentration range of 10^{-5}-10^{-3} M. For determination of the penetration coefficient the following ratio has been used:

$$G_{specific} = \frac{z^2 \cdot F^2}{R \cdot T} \cdot C \cdot P$$

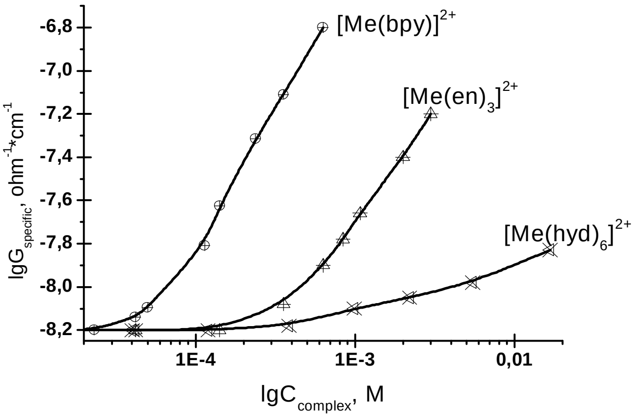

Fig. 6. Dependence of specific membrane conductivity on the concentration of complexes exerting maximal influence on BLM

As is known [22], penetration coefficients through BLM can change in a wide range: from 10^{-3} (for ethanol) to 10^{-14} (for Ca^{2+}) and 10^{-15} for heavy metal cations, respectively. They depend on particle polarity and lipophility. Therefore, we have evaluated lipophility of these complex ions based on the "water/*n*-octanol" distribution coefficient [23, 24].

The obtained results are presented in the table:

	$[Me(bpy)]^{2+}$	$[Me(en)_3]^{2+}$	$[Me(hyd)_6]^{2+}$
P	$(1.2\pm0.2)\cdot 10^{-11}$ cm/s	$(1.96\pm0.2)\cdot 10^{-12}$ cm/s	$(4.26\pm0.2)\cdot 10^{-13}$ cm/s
lgb_i	0.75±0.03	0.5±0.02	-0.2±0.01

As was mentioned above, for bipyridyl and ethylenediamine complexes the nature of central atom does not influence the capability of the complex particles to interact with BLM. The role of the metal ion is to create the most efficient transport form with the necessary quantity of ligands allowing to reach the lipophility and penetrate through the nonpolar BLM matrix. Interaction of hydrazine complexes with BLM testifies to this hypothesis – nickel(II) ions creates more efficient transport form as compared to copper(II) and zinc(II).

Formation of complexes and bonding of a definite number of ligands with the metal ion result in the changing of lipophilic-hydrophilic balance of the ion. It leads to the formation of a water-soluble ion, which, at the same time, has sufficient lipophilicity for interaction with the membrane lipid matrix and is capable of penetrates through BLM. Hence, formations of complex ions with assigned characteristics results in the creation of more convenient transport form, both metal ions and ligands. In the complex structure, the metal ion can penetrate through the membrane and ligand obtains the possibility to move under the influence of the difference of potentials in addition to the concentration gradient influence.

4. Conclusions

This paper is devoted to investigation of "structure-effect" correlation based on interaction of some nitrogen-containing metal complexes with BLM. The possible mechanisms of complex interaction with model cell membrane have been proposed. The role of the conditions (pH, metal and ligand concentration) on the complexes formation and their interaction with BLMs has been determined. It has been also shown that lipophility of a complex particle is a very important factor. All the above can be useful for further purposeful synthesis of bioactive compounds and analysis of their influence on biological objects.

Acknowledgements

We frankly thank to professor M. Cieslak-Golonka (Institute of Inorganic Chemistry and Metallurgy of Rare Elements, University of Technology, Poland) for synthesized and graciously given zinc and copper(II) complexes with bipyridyl.

References

[1] Rosenberg B., VanCamp L. and Trosko J.: Nature, 1969, **222**, 385.

[2]. Koepf-Maier P. and Koepf H.: Chem. Rev., 1987, **87**, 1137.

[3] Bakhtian R. and Ochiai E.: General Pharmacology, 1999, **32**, 525.

[4] Kriss E., Volchenskova I., Grigor'eva A. *et al.*: Koordinatsionnye Soedineniya Metalov v Meditsine. Naukova dumka, Kiyv 1986.

[5] Tien H. and Ottova-Leitmannova A.: Planar Lipid Bilayers (Blms) and their Applications. Elsevier Science Ltd. 2003.

[6] Folch J., Lees M. and Stanley G.: J. Biolog. Chem., 1957, **226**, 497.

[7] Mueller P., Rudin P., Tien M. and Wescit W.: [in:] Danielli J., Pankhurst K. and Riddiford A. (Eds.), Recent Progress in Surface Science. Academic Press, N.Y.-London 1964.

[8] Tien Ti H.: J. Phys. Chem., 1984, **88,** 3172.

[9] Kylyvnyk K. and Ksenzhek O.: Voprosy Khim. i Khim. Technol., 2003, **5**, 124.

[10] Cieslak-Golonka M., Bartecki A. and Jezierska J.: Polyhedron, 1991, **10**, 2179.

[11] Bjerruum J.: Metal Ammine Formation in Aqueous Solution. Theory of the reversible step reactions. P. Hasse & Son, Copenhagen 1957.

[12] Ahmad N. and Rahman S.: Zeitschrift für anorganische und allgemeine Chemie, 1964, **330**, 210.

[13] Kirschenbaum L. and Kustin K.: J. Chem. Soc. A, 1970, **1**, 684.

[14] Cabani S., Moretti G and Scrocco E.: J. Chem. Soc., 1962, **17**, 88.
[15] Cabani S. and Landucci M.: *ibid*, 278.
[16] http://home.tiscali.nl/cheaqs.
[17] Alderighi L., Gans P., Ienco A., *et al*.: Coordination Chem. Rev., 1999, **184**, 311.
[18] Bjerrum J., Schwarzenbach G. and Sillen L.: Stability Constants of Metal-Ion Complexes: Part I Organic Ligands. Burlington House, Chemical Society: London 1975.
[19] Lide D. (Ed.): Handbook of Chemistry and Physics (84th edn.), CRC Press LLC 2004.
[20] Omel'chenko O.: Itogi Nauki i Tekhniki,1991, **29**, 10.
[21] Khmars'ka L., Kylyvnyk K. and Shtemenko O.: Voprosy Khim. i Khim. Technol., 2007, **4**, 41.
[22] Brahm J., Funder J. and Gasbjerg P.: Eleventh School on Biophysics of Membrane Transport, Poland 1992, **1**, 9.
[23] Leo A., Hansch C. and Elkins D.: Chem. Rev., 1971, **71**, 526.
[24] Dataset of LogP values C – logP: BioByte Corp., Claremont, CA, USA.

ВПЛИВ КОМПЛЕКСОУТВОРЕННЯ Ni(II), Cu(II) ТА Zn(II) НА ВЗАЄМОДІЮ З МОДЕЛЬНИМИ КЛІТИННИМИ МЕМБРАНАМИ

Анотація. *Досліджено вплив комплексних сполук нікелю(II), купруму(II) та цинку(II) з деякими нітрогенвмісними лігандами на провідність бішарових ліпідних мембран (БЛМ). Проведена оцінка впливу умов (pH, концентрації йонів металу та лігандів) на комплексоутворення та взаємодію з БЛМ. Визначено склад та форму комплексних йонів у розчині, які максимально змінюють провідність БЛМ. На основі дослідження взаємозв'язку між долею комплексного йону, яка переважає у розчині, та провідністю БЛМ показано, що вплив комплексного йону визначається лігандним оточенням та не залежить від природи центрального атому. Для комплексних йонів, які максимально змінюють провідність БЛМ, були розраховані коефіцієнти проникності.*

Ключові слова: *комплекси металів, нітрогенвмістні ліганди, бішарова ліпідна мембрана (БЛМ), провідність, ліпофільність.*