Non-Crystalline Schiff Base Derivative Complexes with Cu(II) Ions: Molecular Structure Determination with Spectroscopic Techniques

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The binding mechanism between Cu(II) ions and bioactive Schiff base derivative ligands, i.e., 2-[(4-methoxyphenylimino)metyl]phenol, 4,6-dichloro-2-[(4-hydroxyphenyl-imino)methyl]phenol and 4,6-dichloro-2-[(4-methoxyphenylimino)methyl]phenol, in the form of non-crystalline complexes has been successfully studied using the multi-technique methodology based on X-ray absorption fine structure spectroscopy. A distorted square planar geometry around Cu(II) ions composed of two bidently coordinated ligands has been found for all the three complexes. Nitrogen as well as oxygen atoms have been identified as active in the binding process. The final three-dimensional molecular models have been proposed for each of the studied complexes.

topics: XAFS, XANES, EXAFS, bioactivity

1. Introduction

To assess the effectiveness of a country's health system, data on mortality is annually collected by the World Health Organization (WHO). According to a recent WHO report, cancer remains one of the major health problems in both the developed and developing countries. Environmental and behavioral risks together with infectious causes are reported to be responsible for 45% of cancer deaths worldwide. The increasing number of new diagnostic cases has been reported by the International Agency for Research on Cancer [1, 2].

Chemotherapy is one of the basic types of cancer treatment, in addition to surgery, radiation and immunotherapy. Since it uses drugs to kill cancer cells, the basic issue is the adjustment of proper doses — high enough to reduce the risk of drug resistance and causing the minimal side effects. Finding the right balance between the effectiveness of chemotherapeutics and drug resistance or side effects requires a new solution and is a challenge in the field of modern pharmacology [3–5].

Bioactive organic molecules seem to be a promising research direction. Additionally, when coordinated to metal ions, they often show an increase in activity. Our recent studies were focused on the Schiff base derivatives, compounds of the imine class (see Fig. 1). A wide spectrum of antimicrobial activity together with their affinity to coordinate to metal ions makes these systems suitable for applications in the pharmaceutical industry [6–10].

One way to increase the cytotoxic activity of a compound is to substitute an aromatic ring or a halogen atom into its structure. On the other hand, a coordination to metal ions is also very promising [11, 12]. Structural parameters of such a metal–bioactive ligand system, type and number of coordinating ligands next to the complex's coordination geometry usually define its properties. Since the potential of pharmacological action depends on chemical structure, there is an interest in a detailed investigation of the complex's molecular structure. However, obtaining such compounds in the form of good-quality crystals is difficult and in many cases impossible without the use of aggressive solvents which can influence the structure of the complex. Therefore, for the structural investigation of the metal–organic ligand binding site in non-crystalline metal complexes more sophisticated approach is needed due to the fact that diffraction techniques are excluded.

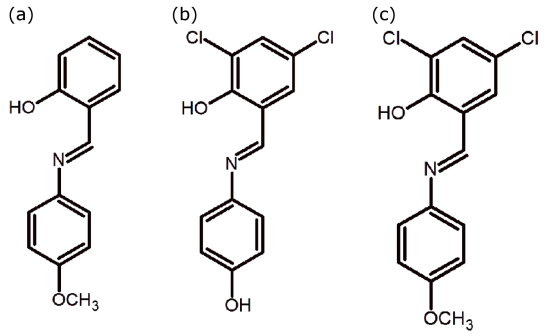

Fig. 1. Salicylideneaniline derivatives used in coordination reaction: (a) 2-[(4-methoxyphenylimino)metyl]phenol, (b) 4,6-dichloro-2-[(4-hydroxyphenylimino)methyl]phenol, (c) 4,6-dichloro-2-[(4-metoksyfenyloimino)metylo]fenol; H1, H2, and H3, respectively.

Biological activity of these compounds, especially of copper(II) complexes, has been widely reported, although the structural data on such complexes has been reported based on methods which cannot provide direct structural information, e.g., IR and UV-VIS spectroscopies, mass spectroscopy or theoretical calculations.

The determination of the coordination geometry around copper ions in reported non-crystalline Schiff-base derivative complexes was carried out based on multi-technique methodology. Such an approach has been successfully used in similar experiments. The basis for the methodology is the X-ray absorption spectroscopy (XAS). The X-ray absorption near the edge structure (XANES) encodes information on a geometric arrangement of atoms in the investigated compound. First, the oxidation state of the absorbing ion may be evaluated, while usually the energy position of the absorption edge is shifted towards higher energies. Second, the comparison of theoretically calculated XANES spectra of modelled complexes with experimental data allows to identify the coordination geometry around the absorber. From the oscillatory part of the spectrum (EXAFS), direct structural information about the type and number of neighboring atoms as well as their distance to the absorbing atom and the Debye–Waller factor may be obtained. Within 2 Å radius around the absorbing ions, the distinction of light elements might be possible, dependently on the system under study, the range and quality of data used for analysis.

XAS combined with spectroscopic and analytical laboratory-based techniques enables us to get knowledge about the molecular structure of the studied complexes [13–18]. Methodology used for the structural characterization of metal complexes is divided into three main steps [17]. First, basic structural characteristics are performed with the determination of the metal to ligand ratio (elemental analysis) and identification of functional groups potentially active in the coordination process (IR spectroscopy). Second, in order to identify the absorber's nearest atomic environment, EXAFS analysis is performed. Third, the geometrical arrangement around the absorber is established with XANES analysis, confirmed with the density functional theory (DFT) calculations and the molecular structure is rechecked with final EXAFS analysis.

2. Experiment

A series of three Cu(II) complexes with cytotoxically active salicylideneaniline derivatives has been synthesized in the Medical University of Warsaw and subsequently cytotoxically evaluated against human pancreatic and cervical cancer cell lines.

A methanolic solution of the Schiff base H1, H2 or H3 (1 mmol) was refluxed with copper(II) acetate (0.5 mmol). The reaction mixture was magnetically stirred (5–7 h). The progress of the reaction was examined by thin layer-chromatography (TLC). After a complete conversion of ligands, the separated Cu(II) complexes (1, 2, and 3) were filtered, washed with methanol, and dried over anhydrous calcium chloride.

The elemental analysis was performed with the Vario El Cube analyzer and content of C, H, N in Cu(II) complexes has been determined. IR spectra have been collected using Thermo Scientific Nicolet iS5 spectrometer in reflectance mode (the range of measurement 400–4000 cm^{-1}). XAS spectra of powder samples have been measured in a transmission mode at Cu K-edge at XAFS beamline of Elettra synchrotron. Data analysis has been carried out with the IFFEFIT package [19, 20] and FEFF 9.6 code [21]. Ground state molecular structures have been optimized at the density-functional theory (DFT) level with the B3LYP exchange-correlation functional and 6-311+G(d,p) basis set. All the calculations were performed using the Spartan'16 software [22–24].

3. Results

3.1. Basic structural characterization

The analysis of the fingerprint area of the Fourier transform infrared (FTIR) spectra, collected for ligands (H1, H2, H3) and corresponding complexes (1, 2, 3), confirmed that Cu(II) complexes have been obtained.

The energy position of the absorption edge for all complexes agrees with +2 oxidation states of copper ions. The comparison was made for the maximum of the first derivative, with CuO and Cu_2O as a reference (see Fig. 2). Moreover, based on EXAFS analysis, oxygen and nitrogen atoms have been found in the nearest atomic environment of Cu(II) ions, about 1.9 Å and 2.0 Å, respectively (see Table I). An iminic nitrogen atom stands as an obvious donor of a free electron pair

TABLE I

EXAFS fitting parameters: distance — R, number of atoms — N, Debye–Waller factor — σ^2 and R-factor.

Complex	Bond	R [Å]	N	σ^2 [Å^2]	R-factor
1	Cu–O	1.89(1)	2	0.003(1)	0.01
	Cu–N	1.97(3)	2	0.003(1)	
	Cu–C_1	2.85(3)	4	0.006(2)	
	Cu–C_2	3.01(1)	2	0.006(2)	
	Cu–C_3	3.36(7)	2	0.006(2)	
2	Cu–O	1.90(1)	2	0.003(1)	0.02
	Cu–N	2.00(1)	2	0.003(1)	
	Cu–C_1	2.89(1)	6	0.008(3)	
	Cu–C_2	3.26(2)	2	0.008(3)	
3	Cu–O	1.90(1)	2	0.004(1)	0.01
	Cu–N	1.99(1)	2	0.004(1)	
	Cu–C_1	2.93(2)	6	0.008(2)	
	Cu–C_2	3.37(5)	2	0.008(2)	

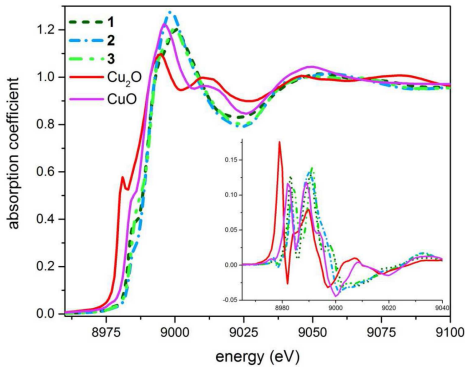

Fig. 2. Analysis of absorption edge energy position for Cu(II) complexes and reference oxides. As an inset — first derivatives, respectively.

during the coordination process, while the coordination process may also proceed through the deprotonated hydroxyl group of a phenolic substituent [13–17, 25–29]. With the elemental analysis, the metal to ligand ratio 1:2 has been determined (see Table II).

3.2. Initial structural model

EXAFS analysis in 3.5 Å radius was performed for the identification of further atomic environment around copper ions. It revealed that further atomic environment of each studied complex is composed of eight carbon atoms. In the case of complex 1, at the distances of about 2.9 Å — four, 3.0 Å — two, and 3.3 Å — two carbon atoms have been found. For chlorine substituted complexes 2 and 3, six and two carbon atoms have been found at the distances of about 2.9 Å and 3.3 Å, respectively (see Table II). Based on these data, molecular models of the studied complexes have been proposed (see Fig. 3) and next refined.

TABLE II

Elemental analysis results. Composition of CHN found for complexes 1, 2, 3 together with values calculated for $Cu(H1)2 \cdot H_2O$, $Cu(H2)2 \cdot 4H_2O$, $Cu(H3)2 \cdot H_2O$, respectively.

Compound	%C, %H, %N	
	Found	Calculated
1	62.74, 4.60, 5.19	62.97, 4.91, 5.25
2	44.55, 2.84, 3.29	44.75, 3.47, 4.01
3	50.78, 2.79, 4.24	50.93, 3.24, 4.10

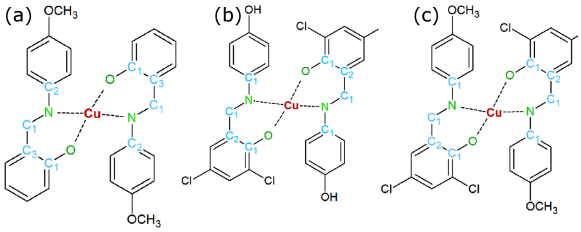

Fig. 3. Initial structural models of complexes 1, 2, 3 in (a), (b), (c), respectively. The nearest and further atomic environment determined with EXAFS analysis is marked.

3.3. Model refinement

For the initial structural models, XANES calculations have been performed. Then, the calculated and experimental spectra have been compared in order to determine the coordination geometry around Cu(II) ions (see Fig. 4). In the case of all complexes, the geometry of distorted flat square, formed around Cu(II) ions by bidentate ligands, reproduces the shape of the experimental XANES spectra. Differences in angle values between Cu–O and Cu–N bonds — 94°, 97°, 95° for complex 1, 2, and 3, respectively — affected the white line shape. Finally, optimized structures have been rechecked with EXAFS analysis for confirmation. Single as well as multiple scattering paths have been included. Fitting results are depicted in Fig. 5.

3.4. Cytotoxic evaluation

A modification in the cytotoxicity profile after complexation has been observed for all Cu(II) complexes (see Fig. 6). The enhanced cytotoxic effect with increasing concentrations of all tested compounds has been observed for both cell lines. In the case of human pancreatic cell lines, compounds with 1 μM concentration weakly affected viability of cells, with viability near 100% in all cases. In turn, for 100 μM concentrations, viability decreases below 25% in almost all cases. Only ligand H1 seems to have no effect on cancer cells in the range of the tested concentrations. In the case of cervical cancer cell lines, the cytotoxic effect strongly affected viability already at the concentration 1 μM, oscillating around 50%. But the

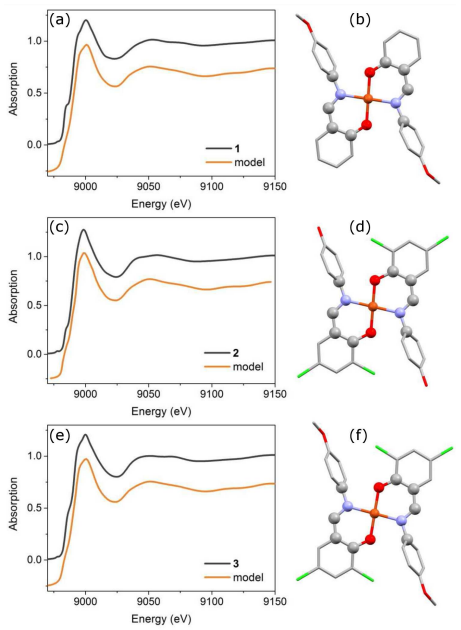

Fig. 4. Comparison of XANES spectra: experimental (black line) and calculated with FEFF 9.6 code (orange line) spectra for complexes 1 in (a), 2 in (c), 3 in (e); three-dimensional molecular structure of studied complexes 1 in (b), 2 in (d), 3 in (f).

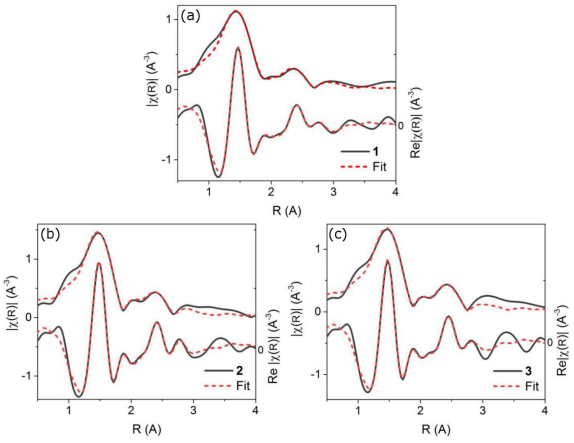

Fig. 5. Moduli of the Fourier transform and the real part of the EXAFS oscillations, together with the final fitting of complexes 1, 2, and 3 in (a), (b), and (c), respectively.

downward trend is milder than for CF-PAC cells, resulting with viability over 25% for H1, H3, and 3 compounds. For H1, viability stands over 50% in the range of the tested concentrations. The highest cytotoxic effect against HeLa cells occurred for H2 and 2 compounds. It seems reasonable to state that the cytotoxic effect is a derivative of the molecular structure of the ligand and the method of its coordination to copper ions. Further studies on this matter are planned. However, at this point it is crucial to know the molecular structure of the studied complexes which is nontrivial in the case of noncrystalline components.

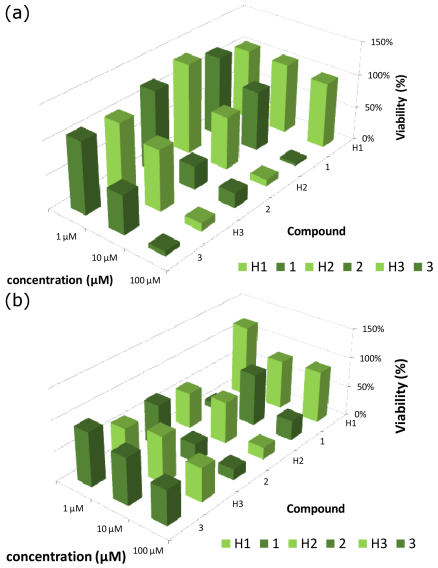

Fig. 6. Cytotoxic activity evaluation results for Cu(II) Schiff base derivative complexes (1, 2, 3) and their parent ligands (H1, H2, H3) — cell viability (%), tested for concentrations 1, 10 and 100 μM. In the cytotoxic evaluation were used: (a) human pancreatic (CFPAC-1) and (b) cervical (HeLa) cell lines. Mouse fibroblasts (NIH3T3) were used as a model of normal cells.

4. Conclusions

Three bioactive Schiff-base derivative complexes with Cu(II) ions have been studied. Non-crystalline compounds have been structurally characterized using multi-technique methodology and molecular structural models have been proposed. For all complexes, the coordination number is four, containing two nitrogen atoms and two oxygen atoms, derived from ligands' iminic nitrogen and phenolic oxygen of a deprotonated hydroxyl group. With the metal to ligand ratio 1:2, molecular models have been proposed. The coordination of the distorted square planar geometry, formed by two bidentate ligands, has been confirmed with XANES calculations for all complexes, wherein the distortion is associated with the angle value between Cu–N and Cu–O bonds. Final EXAFS analysis, including geometrically sensitive multiple scattering paths, confirmed the molecular structure of the studied complexes. A modification in the cytotoxicity profile after coordination to metal ions has been observed. A variation in the potency of cytotoxic action in relation to coordination geometry and complexes' concentration has been noted as well.

Acknowledgments

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