Copper(II) Complexes Containing Schiff Bases Derived from Salicylaldehyde and Esters of L-Glutamic Acid

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Dedicated to RNDr. Miroslav Zikmund, CSc., in honour of his 80th birthday

Copper(II) complexes of composition Cu(TSB)X, where TSB represents tridentate Schiff base derived from salicylaldehyde and L-glutamic acid as well as its methyl and ethyl esters, and X represents imidazole, 2-methylimidazole, 4-methylimidazole, and 2-ethylimidazole were synthesized and studied. Substances in question have been found to adopt square pyramidal arrangement of ligands, and from this point of view they could be taken as model substances simulating the role of natural metalloenzyme Cu,Zn—SOD in living systems. The complexes prepared exhibit significant efficacy against bacteria *Staphylococcus aureus* and possess also antiradical activity. The mutual influence of anionic (TSB) and neutral *N*-donor ligands (X) on their chemical and biological properties is discussed.

The copper remarkable ability to form complexes or chelates with various organic substances is well known. In living systems such complexes provide its distribution and absorption and also many physiological processes are in close connection with copper presence. As it was published [1] many copper(II) complexes have been observed to possess various biological activities of potential therapeutical applications. The relevance of their practical use depends of course on right combination of their kinetic and thermodynamic properties, oxidation state of central atom as well as on number of coordinated ligands, and type of coordination polyhedron they form. In recent years the interest concerning copper(II) complexes has been focused on complexes containing Schiff bases derived from salicylaldehyde and various amino acids. This group of copper(II) complexes was studied due to their found antimicrobial [2—6] and radioprotective activities [7]. In our previous works the copper(II) complexes containing Schiff bases derived from salicylaldehyde and L-glutamic acid [5, 8], or its esterified forms [6, 9] were reported. As additional molecular ligands pyrazole, imidazole, pyridine, and some of their derivatives were used [5, 8]. To study this problem in more detail we found it interesting to prepare the series of copper(II) complexes containing Schiff bases derived from salicylaldehyde and L-glutamic acid (and its methyl and ethyl esters) and to discuss the mutual influence of anionic and molecular ligands on their chemical and biological properties. As molecular ligands imidazole, 2-methylimidazole, 4-methylimidazole, and 2-ethylimidazole were chosen.

EXPERIMENTAL

Copper(II) complexes of composition $[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]\cdot H_2O$ and Cu(sal-L-glu)X, where (sal-L-glu) represents tridentate Schiff base derived from salicylaldehyde and L-glutamic acid and X = imidazole (im), 2-methylimidazole (2-Meim), 4-methylimidazole (4-Meim), and 2-ethylimidazole (2-Etim), were synthesized as described in [5].

The elemental analysis was carried out using an analyzer EA 1108 (Carlo Erba Instruments, Italy) based on chromatographic principle at temperature 1800 °C and in the atmosphere of helium.

The electronic absorption spectra were taken with spectrophotometer Specord M 40 in the $\tilde{\nu}$ range 11 000—30 000 cm^{-1} using nujol mull technique.

For antimicrobial activity evaluation the microorganisms *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* were used. The studied substances were tested in medium of dimethyl sulfoxide (DMSO) the antimicrobial activity of which was found to be 32 000 mmol dm^{-3}. As cultivation media the nutrient broth and nutrient agar for bacteria and Sabouraud medium and Sabouraud agar for fungi were used.

Cu(sal-5-met-L-glu)$(H_2O)_2$ (*a*)
Cu(sal-5-et-L-glu)$(H_2O)_2$ (*b*)

Copper(II) acetate (6.8 g; 0.034 mol) was dissolved in 140 cm^3 of water and the formed bluish green solution was heated to 60—65 °C. Under continuous stirring salicylaldehyde (4.3 g; 0.034 mol) and then 5-

methyl ester of L-glutamic acid (*a*) or 5-ethyl ester of L-glutamic acid (*b*) (5.0 g; 0.034 mol) were added. The colour of solution after 30 min under heating turned to green and in 24 h dark green (*a*) or light green (*b*) products crystallized. The crystals were isolated, washed out with cold water and air-dried.

For $CuC_{13}H_{17}O_7N$ (*a*) (M_r = 362.84) w_i(calc.): 43.03 % C, 3.86 % N, 4.73 % H; w_i (found): 43.71 % C, 3.94 % N, 4.69 % H.

For $CuC_{14}H_{19}O_7N$ (*b*) (M_r = 376.87) w_i(calc.): 44.62 % C, 3.72 % N, 5.09 % H; w_i(found): 44.82 % C, 3.92 % N, 4.96 % H.

Cu(sal-5-met-L-glu)(im)

Cu(sal-5-met-L-glu)$(H_2O)_2$ (1.85 g; 0.005 mol) was dissolved in 9 cm^3 of propanol at 60 °C. The formed solution was mixed with imidazole (0.98 g; 0.015 mol) in 12.5 cm^3 of ethanol. After some days deposited green-blue product was isolated, washed out with small amount of propanol and air-dried.

For $CuC_{16}H_{17}O_5N_3$ (M_r = 394.87) w_i(calc.): 48.67 % C, 10.64 % N, 4.34 % H; w_i(found): 49.06 % C, 10.91 % N, 4.37 % H.

Cu(sal-5-et-L-glu)(im)

Cu(sal-5-et-L-glu)$(H_2O)_2$ (1.85 g; 0.005 mol) was dissolved in 9 cm^3 of propanol at 60 °C. To the formed solution imidazole (0.65 g; 0.01 mol) in 12.5 cm^3 of ethanol was added. After some days deposited grey-green product was washed out with small amount of propanol and air-dried.

For $CuC_{17}H_{19}O_5N_3$ (M_r = 408.90) w_i(calc): 49.94 % C, 10.28 % N, 4.69 % H; w_i(found): 50.28 % C, 10.17 % N, 5.02 % H.

Cu(sal-5-met-L-glu)(2-Meim)

Cu(sal-5-met-L-glu)$(H_2O)_2$ (1.85 g; 0.005 mol) was dissolved in 9 cm^3 of propanol at 60 °C and then mixed with 2-methylimidazole (1.00 g; 0.015 mol) in 12.5 cm^3 of ethanol. After two days crystallized bluish green product was isolated, washed out with small amount of propanol and air-dried.

For $CuC_{17}H_{19}O_5N_3$ (M_r = 408.83) w_i(calc.): 49.92 % C, 10.27 % N, 4.69 % H; w_i(found): 48.47 % C, 10.65 % N, 4.58 % H.

Cu(sal-5-et-L-glu)(2-Meim)

Cu(sal-5-et-L-glu)$(H_2O)_2$ (1.85 g; 0.005 mol) was dissolved in 9 cm^3 of propanol (t = 60 °C) and mixed with 2-methylimidazole (0.67 g; 0.01 mol) in 12.5 cm^3 of ethanol. After two days deposited product was washed out with small amount of propanol and air-dried.

For $CuC_{18}H_{21}O_5N_3$ (M_r = 422.86) w_i(calc.): 51.12 % C, 9.94 % N, 5.02 % H; w_i(found): 51.32 % C, 10.29 % N, 5.24 % H.

Cu(sal-5-met-L-glu)(4-Meim) (*a*)
Cu(sal-5-et-L-glu)(4-Meim) (*b*)

Cu(sal-5-met-L-glu)$(H_2O)_2$ (*a*) or Cu(sal-5-et-L-glu)$(H_2O)_2$ (*b*) (1.85 g; 0.005 mol) dissolved in 9 cm^3 of propanol at 60 °C was mixed with 4-methylimidazole (0.82 g; 0.01 mol) in 12.5 cm^3 of ethanol. After some days crystallized grey-green products were isolated, washed out with small amount of ethanol and air-dried.

For $CuC_{17}H_{19}O_5N_3$ (*a*) (M_r = 408.83) w_i(calc.): 49.94 % C, 10.28 % N, 4.69 % H; w_i(found): 50.58 % C, 10.93 % N, 5.05 % H.

For $CuC_{18}H_{21}O_5N_3$ (*b*) (M_r = 422.86) w_i(calc.): 51.12 % C, 9.94 % N, 5.01 % H; w_i(found): 50.79 % C, 10.18 % N, 5.24 % H.

Cu(sal-5-met-L-glu)(2-Etim) (*a*)
Cu(sal-5-et-L-glu)(2-Etim) (*b*)

Cu(sal-5-met-L-glu)$(H_2O)_2$ (*a*) or Cu(sal-5-et-L-glu)$(H_2O)_2$ (*b*) (1.85 g; 0.005 mol) dissolved in 9 cm^3 of propanol at 60 °C was mixed with 2-ethylimidazole (0.96 g; 0.01 mol) in 12.5 cm^3 of propanol. After some days the greyish green (*a*) or bluish green (*b*) product was isolated, washed out with ethanol and air-dried.

For $CuC_{18}H_{21}O_5N_3$ (*a*) (M_r = 422.96) w_i(calc).: 51.11 % C, 9.94 % N, 5.01 % H; w_i(found): 51.78 % C, 10.12 % N, 5.22 % H.

For $CuC_{19}H_{23}O_5N_3$ (*b*) (M_r = 436.99) w_i(calc.): 52.22 % C, 9.62 % N, 5.31 % H; w_i(found): 52.89 % C, 10.18 % N, 5.62 % H.

DISCUSSION

As it was published elsewhere [10, 11] one of two carboxylic groups in L-glutamic acid stays uncoordinated even after complexation and modification of this group is expected to influence some properties of such type complexes, *e.g.* solubility. On the basis of it the series of copper(II) complexes of composition Cu(TSB)X, where TSB = tridentate Schiff base derived from salicylaldehyde and L-glutamic acid (as well as its methyl and ethyl esters) and X represents imidazole, 2-methylimidazole, 4-methylimidazole, and 2-ethylimidazole was synthesized with the aim to study and to correlate their chemical and biological properties.

For characterization of substances prepared the visible spectral data listed in Table 1 were used. The found positions of maxima located in the $\tilde{\nu}$ range 15 500—17 500 cm^{-1} are indicative of square planar coordination of components around the central Cu(II) atom and the maxima in the $\tilde{\nu}$ range 25 000—28 000 cm^{-1} could be assigned to intraligand transi-

Table 1. Electronic Spectral Data of Cu(II) Complexes Studied ($\tilde{\nu}_i/\mathrm{cm}^{-1}$)

Complex	$\tilde{\nu}_{1\,\max}$	$\tilde{\nu}_{2\,\max}$
$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2] \cdot H_2O$	15440	26440
$Cu(sal\text{-}5\text{-}met\text{-}L\text{-}glu)(H_2O)_2$	15640	26240
$Cu(sal\text{-}5\text{-}et\text{-}L\text{-}glu)(H_2O)_2$	15720	26240
Cu(sal-L-glu)(im)	15720	28040
Cu(sal-5-met-L-glu)(im)	16880	26200
Cu(sal-5-et-L-glu)(im)	16880	25760
Cu(sal-L-glu)(2-Meim)	17240	26480
Cu(sal-5-met-L-glu)(2-Meim)	16120	26000
Cu(sal-5-et-L-glu)(2-Meim)	16200	26720
Cu(sal-L-glu)(4-Meim)	16520	26480
Cu(sal-5-met-L-glu)(4-Meim)	17240	25280
Cu(sal-5-et-L-glu)(4-Meim)	17520	25480
Cu(sal-L-glu)(2-Etim)	17400	27000
Cu(sal-5-met-L-glu)(2-Etim)	16240	24960
Cu(sal-5-et-L-glu)(2-Etim)	16640	25720

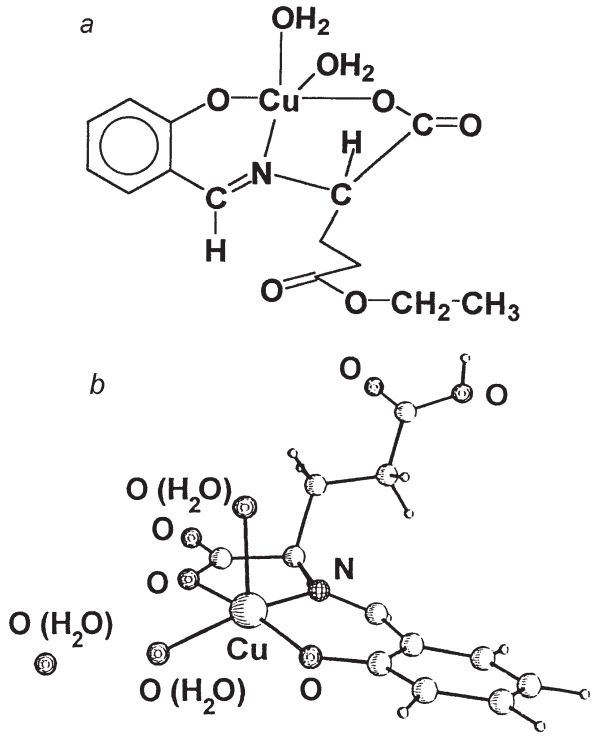

Fig. 1. The assumed scheme of structural arrangement in copper(II) complexes containing esterified form of L-glutamic acid (*a*) based on solved molecular structure of $[Cu(sal\text{-}L\text{-}glu)(H_2O)_2] \cdot H_2O$ (*b*) [12].

tions. As it is evident no significant influence of anionic as well as neutral ligands on their positions was observed. Thus it is reasonable to assume that all complexes under study adopt square pyramidal arrangements of ligands as it was stated for the used parent complex containing water as molecular ligand $[Cu(sal\text{-}L\text{-}glu)(H_2O)_2] \cdot H_2O$ [12]. In accordance with authors' data the tridentate Schiff base is coordinated in basal plane of square pyramid *via* phenolic and carboxylic oxygens and azomethine nitrogen and the fourth site as well as the apical position of pyramid are occupied by *O*-donor atoms of water molecules. On the basis of these facts it could be concluded that in Cu(II) complexes prepared the water molecule coordinated in basal plane of pyramid is replaced by *N*-donor atom of corresponding molecular ligand (imidazole and derivatives) under changing of Cu(II) chromophore [Cu, O—N—O, O] to [Cu, O—N—O, N] one. The apical position of pyramid is then assumed to be occupied by carboxylic oxygen from an adjacent complex unit. The proposed scheme of structural arrangement of individual components in complexes with esterified form of L-glutamic acid, based on solved structure of $[Cu(sal\text{-}L\text{-}glu)(H_2O)_2] \cdot H_2O$ [12], is shown in Fig. 1.

The antimicrobial activity of investigated copper(II) complexes was evaluated using bacteria *Staphylococcus aureus*, *Escherichia coli* and fungi *Candida albicans* under conditions mentioned in Experimental. The results (listed in Table 2) are presented in terms of minimal inhibition concentration (MIC), representing the minimum concentration of substances tested (mmol dm^{-3}) inactivating the microorganism reproducibility. As it can be seen from Table 2 the studied Cu(II) complexes exhibit the highest efficacy against bacteria *Staphylococcus aureus* and it is evident that the presence of methyl and/or ethyl groups in L-glutamic acid increases the inhibition ability almost of all substances tested, regardless of coordinated molecular ligand.

The observed square pyramidal coordination of copper(II) [5, 12] and nature of donor ligands in complexes presented are very close to constitution of active centre of natural enzyme superoxide dismutase, Cu,Zn—SOD. As it was reported elsewhere [1, 13, 14] the Cu(II) ion in SOD molecule is also pentacoordinated with square pyramidal arrangement of components. The four planar sites are occupied by *N*-donor atoms of histidine and the fifth apical position by the water molecule. From this structural point of view the copper(II) complexes of this type might serve as simple models simulating the role of this enzyme in living systems. According to in recent years published data shown in Table 3 the ability of complexes prepared to imitate SOD enzyme activity was proved. On the basis of listed data it can be concluded that methyl and ethyl groups in esterified forms of L-glutamic acid are without influence on the value of IC_{50}, but on the other hand, the derivatives of imidazole evidently improved this ability from 10^{-6} to 10^{-7} mol dm^{-3}. Even when the investigated copper(II) complexes did not reach the activity of natural enzyme (10^{-9} mol dm^{-3}) the advantage of them is in their 100 lower relative molecular mass allowing their better transport through cellular membranes [1].

Table 2. Antimicrobial Activity of Copper(II) Complexes Studied (mmol dm^{-3})

Complex	*Staph. aureus*	*Escherichia coli*	*Candida albicans*
$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2] \cdot H_2O$	0.425	3.408	3.408
$Cu(sal\text{-}5\text{-}met\text{-}L\text{-}glu)(H_2O)_2$	0.215	3.445	3.445
$Cu(sal\text{-}5\text{-}et\text{-}L\text{-}glu)(H_2O)_2$	0.207	3.317	3.317
Cu(sal-L-glu)(im)	0.205	3.285	3.285
Cu(sal-5-met-L-glu)(im)	0.198	3.166	3.166
Cu(sal-5-et-L-glu)(im)	0.191	3.057	3.057
Cu(sal-L-glu)(2-Meim)	0.198	2.956	3.165
Cu(sal-5-met-L-glu)(2-Meim)	0.191	3.165	3.057
Cu(sal-5-et-L-glu)(2-Meim)	0.092	3.057	2.956
Cu(sal-L-glu)(4-Meim)	0.396	2.956	3.165
Cu(sal-5-met-L-glu)(4-Meim)	0.764	3.057	6.114
Cu(sal-5-et-L-glu)(4-Meim)	0.185	2.956	5.912
Cu(sal-L-glu)(2-Etim)	0.762	3.050	3.050
Cu(sal-5-met-L-glu)(2-Etim)	0.184	2.955	2.955
Cu(sal-5-et-L-glu)(2-Etim)	0.357	2.861	2.861

Table 3. The SOD Mimetic Activity of Some Representative Substances of Copper(II) Complexes Prepared Presented in Terms of Values IC_{50} and $-\log\{IC_{50}\}$, Determined either by Photochemical (f) or Enzymatic (e) Methods [1]

Complex	$IC_{50}/(mol\ dm^{-3})$	$-\log\{IC_{50}\}$	Ref.
$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2] \cdot H_2O$	2.38×10^{-6} (e)	5.62	[8]
	2.11×10^{-6} (f)	5.67	[15]
Cu(sal-L-glu)(im)	1.31×10^{-6} (e)	5.88	[8]
Cu(sal-L-glu)(2-Meim)	1.57×10^{-7} (f)	6.80	[15]
Cu(sal-L-glu)(4-Meim)	3.93×10^{-7} (f)	6.40	[15]
$Cu(sal\text{-}5\text{-}et\text{-}L\text{-}glu)(H_2O)_2$	1.84×10^{-6} (e)	5.74	[16]
	1.68×10^{-6} (f)	5.77	[16]
Cu(sal-5-et-L-glu)(im)	2.92×10^{-6} (e)	5.53	[16]
Cu,Zn—SOD	3.29×10^{-9} (e)	8.48	[8]

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