

On active sites of N-salicylideneaminoacidato copper(II) complex in basic solutions

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In the memory of Prof. Ing. Peter Pelikán, DrSc. (1939-2002)

Abstract

The geometries of (imidazole- N^3)(N-salicylidene-alaninato-O,N,O') copper(II) complex (CuSalala.imid) and of its possible adducts with OH^- and H_2O at various sites of CuO_2N_2 coordination polyhedron have been optimized using B3LYP hybrid functional. OH^- anion bonded at Cu site replaces the imidazole ligand in the copper (II) coordination sphere. This may be understood as the first step of experimentally observed copper (II)hydroxide formation in the absence of reducing sugars. Stable adducts with H_2O have been found at oxygen sites only. Their formation is less energetically advantageous due to hydrogen bonding only. Water molecules activate CuSalala.imid by weakening the Cu-O bonds.

Key words: Blue protein model compounds; DFT method; Electronic structure; Optimal geometry;

Introduction

(Imidazole- N^3)(N-salicylidene-alaninato-O,N,O') copper(II) complex (CuSalala.imid – see Fig. 1) as the model system of galactose oxidase was the object of our previous quantum-chemical studies (Breza 2006a, 2006b). Galactose oxidase is a mononuclear copper protein which catalyses the two-electron oxidation of primary alcohols to aldehydes with subsequent reduction of dioxygen to peroxide (Whittaker 1988, 1989, 1990, 2003, Wang 1996, Halfen 1997). Its active site involves four amino acid ligands (O atoms from Tyr₂₇₂ and Tyr₄₉₅, N atoms from His₄₉₆ and His₅₈₁) directly coordinated to a mononuclear Cu center in the protein which is also bound by a solvent molecule to form a distorted five-coordinate metal complex. There are three distinct redox forms associated with two one-electron oxidation-reduction

steps: The fully oxidized catalytically active (AGO) form is green, the fully reduced (RGO) form is colorless and the catalytically inactive (IAGO) intermediate is blue.

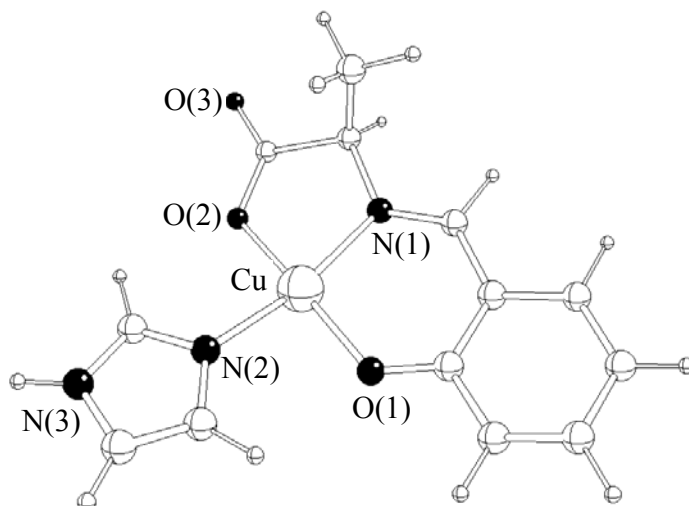


Fig. 1 Optimized geometry of neutral CuSalala.imid molecule (model A)

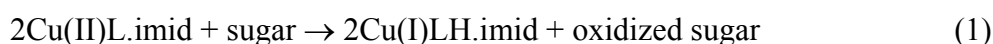
Mockler and coworkers (Mockler 1999, 2003, private communication, Butcher 2004) have studied catalytic properties of a series of *N*-salicylideneaminoacidato copper(II) complexes with imidazole derivatives (Cu(II)L.imid). In the presence of sodium hydroxide, various sugars (such as galactose, glucose, fructose, mannose, maltose but not sucrose) are oxidized by CuL.imid in methanol/water and acetonitrile/water solutions. In methanol solution it can oxidize a ten-fold excess of sugar with the low solubility of the sugars in methanol appearing to limit the degree of catalytic activity.

When an aqueous solution of sodium hydroxide was added to Cu(II)L.imid dissolved in acetonitrile or methanol, blue green copper(II) hydroxide slowly precipitated from green solution over a period of several days. There is no evidence that the complexes oxidize methanol under these conditions (Mockler 1999, 2003, private communication, Butcher 2004).

When an aqueous sodium hydroxide solution of a sugar is added to Cu(II)L.imid dissolved in acetonitrile or methanol the green solution turns yellow with the formation of a white precipitate over a period of 6-24 hours. If the solutions are placed in a sonicator the reaction takes place in less than 40 minutes. Electron spectra indicate that the copper atom remains in a square pyramidal environment with the imidazole coordinated to the copper atom similarly as in solid state. There is little, if any, change in the spectra when a sugar is

added to the solution, suggesting that either the sugar does not bind to the copper atom in solution or that an oxygen ligand atom is replaced by a sugar oxygen atom of similar ligand field strength (Mockler 1999, 2003, private communication, Butcher 2004).

Based on negative ion electrospray ionisation mass spectra Mockler concluded that the following reaction has occurred (Mockler 1999, 2003, private communication, Butcher 2004)



where L denotes N-salicylidene-aminoacidate anionic ligand. As the existence of acidic Cu(I)LH.imid compound in basic solutions is improbable, the above mentioned yellow solution corresponds to an unknown intermediate Cu(I) complex. Thus, the Eq. (1) should be understood as the simplified description of the whole process only. The true reaction mechanism can be suggested on the basis of more complex treatment, including quantum-chemical modeling the structures of possible reaction intermediates (Breza 2006b).

An ab initio computational study (Garcia-Raso, 2001) of the possible transition states has been performed in order to evaluate the mechanism (associative versus dissociative) in the formation of various N-salicylidene-aminoacidato copper(II) ternary complexes with 2-aminopyridine (or pyrimidine). Due to technical reasons, 3-hydroxypropenal instead of N-salicylidene and guanidine instead of 2-aminopyridine moieties have been used in the simplified model system. Electronic structure of aqua(N-salicylidene-methylester-L-glutamato)Cu(II) monohydrate (Langer 2003), (N-salicylidene-D,L-glutamato)(2-methylimidazole) copper(II) (Langer 2004b) and aqua(N-salicylidene-methylester-L-glutamato)Cu(II) monohydrate (Langer 2004a) has been investigated at B3LYP/SVP level of theory in the geometries derived from the experimental ones.

In our previous studies (Breza 2006a) the total charge (and spin) dependence of optimal geometry and electronic structure of CuSalala.imid has been investigated at B3LYP/6-31G* level of theory. Its energy significantly rises with the electron removal and the oxidation of a neutral molecule demands much more energy (over 160 kcal/mol) than of its anion (ca 10 kcal/mol). Thus the existence of the third fully oxidized form of this compound is improbable and the catalytic mechanism of sugars oxidation proposed for the galactose oxidase (Whittaker 1988, 1989, 1990, 2003, Wang 1996, Halfen 1997) cannot be used in our system. Copper d electron populations (from 9.4 to 9 electrons) indicate the highest probability of Cu(II) oxidation state in all the systems under study.

For the formation of the intermediate complexes with sugars/alcohols, the addition of their oxygen (to the sites with high nucleophilic reactivity index) and hydrogen (to the sites with high electrophilic reactivity index) atoms may be supposed. The interaction of O_{sugar} with Cu and hydroxyl H_{sugar} with some of Cu neighbours may be expected in the adduct. Fukui reactivity indices of these atoms in CuSalala.imid (Breza 2006b) obtained at B3LYP/6-31G* level of theory predict the highest probability of Cu... O_{sugar} , alaninate N(1) ... O_{sugar} and phenoxyl O(1) H_{sugar} interactions. In the next step the hypothetical reduced form of CuSalala.imid complex was modeled (Breza 2006b). Nevertheless, our B3LYP/6-31G* energy data indicate the highest stability of the neutral CuSalalaH.imid structures with an additional hydrogen bonded to some of carboxyl oxygens with released (or significantly weakened) Cu–O(carboxyl) bond.

As mentioned above, the formation of such an acidic structure in basic solutions is highly improbable. The formation of CuSalala.imid adducts with OH^- and H_2O species as the first step of the reaction pathway is more probable. The aim of this quantum-chemical study is to investigate the possible structures of these adducts. For the sake of simplicity, we will restrict to the most probable reaction sites at Cu and its neighbour atoms.

Method

The geometries of the neutral CuSalala.imid (Fig. 1) and of its adducts with OH^- or H_2O located at Cu, O(1), O(2), N(1) or N(2) site are optimized at B3LYP level of theory using the Gaussian98 program package (Frisch 1998). For Cu, N and O atoms, 6-31+G* basis sets from the Gaussian basis set library are used whereas the 6-31G* ones are used for C and H atoms. Hydrogen atoms of OH^- and H_2O are augmented with one diffusion s function (exponent of 0.036). The obtained structures stability is confirmed by vibration analysis. The zero-point energy (ZPE) correction and Gibbs free energy at room temperature (G_{298}) are evaluated as well. The electronic structure of the systems under study is evaluated in terms of Mulliken population analysis (MPA) using 6-31G basis sets for all atoms. Alternatively, Natural Bond Orbitals (NBO) population analysis (Carpenter 1988) using original basis sets is utilized for this purpose. MOLDRAW software (Ugliengo 1993) is used for geometry manipulations and visualization purposes.

Results and Discussion

In the first step, CuSalala.imid geometry obtained in (Breza 2006b) has been reoptimized in new basis set (model A, see Fig. 1). In the next step, OH⁻ or H₂O species were located in the vicinity (at the distance of ca 2 Å) of its Cu, O(1), O(2), N(1) or N(2) sites. After the geometry optimization, only three stable structures have been obtained - one with OH⁻ (model B, see Fig. 2) and two with H₂O (models C1 and C2, see Figs. 3 and 4).

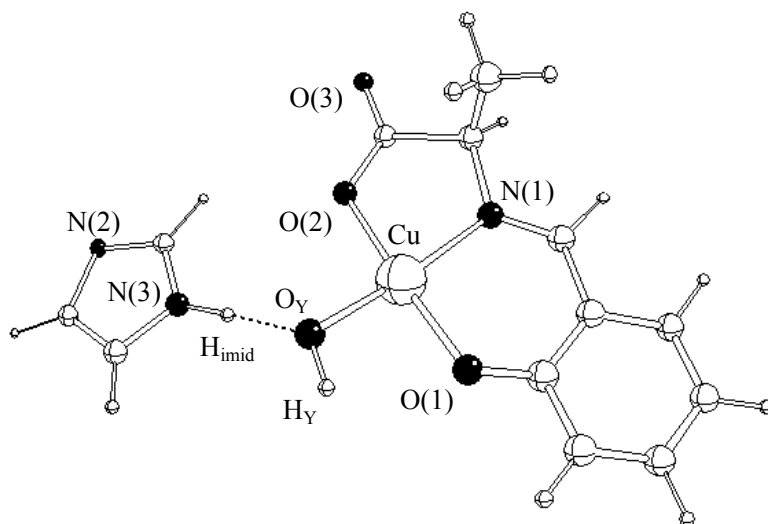


Fig. 2 Optimized geometry of CuSalala.OH⁻...imid adduct (model B)

Nucleophilic OH⁻ species bonded at Cu site (model B) causes the Cu-N(2) bond split and replaces the imidazole ligand in Cu coordination sphere. Energy data (Table 1) indicate exothermic character of this replacement reaction which may be understood as the first step of experimentally observed copper(II) hydroxide formation in the absence of sugar. We have not found any OH⁻ adduct at N(1) site predicted by Fukui reaction indices (Breza 2006b). The formation of CuSalala.imid adducts with H₂O at O(1) (model C1) and O(2) (model C2) sites is not so energetically advantageous (Table 1) due to hydrogen bonding formation only. The model C2 is more stabilized by the stronger interaction of water oxygen (O_Y) with a hydrogen of imidazole (H_{imid}) ligand. Thus it is more stable than the C1 one which might be unstable at room temperature as indicated by Gibbs free energy value (Table 1). Nevertheless, a solvent effect should be considered in the calculations to obtain more reliable data.

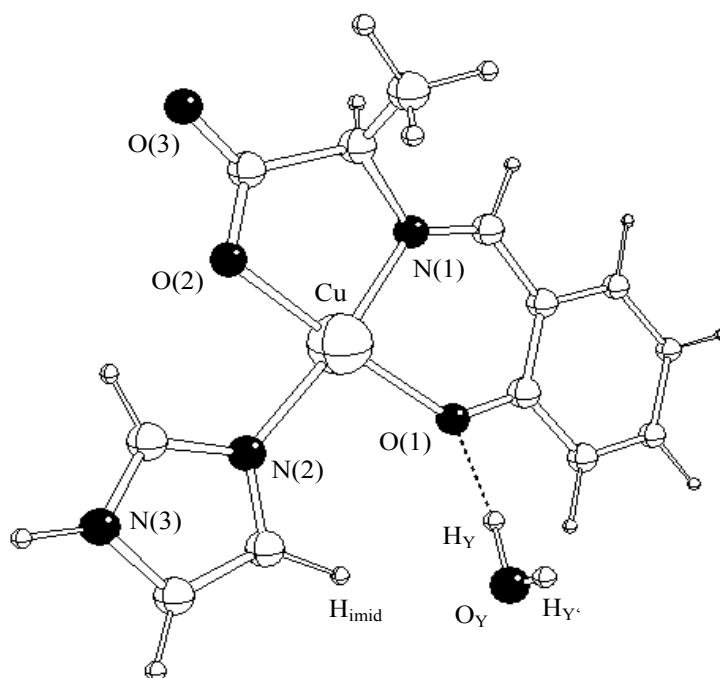


Fig. 3 Optimized geometry of CuSalala.imid...H₂O adduct hydrated at O(1) site (model C1)

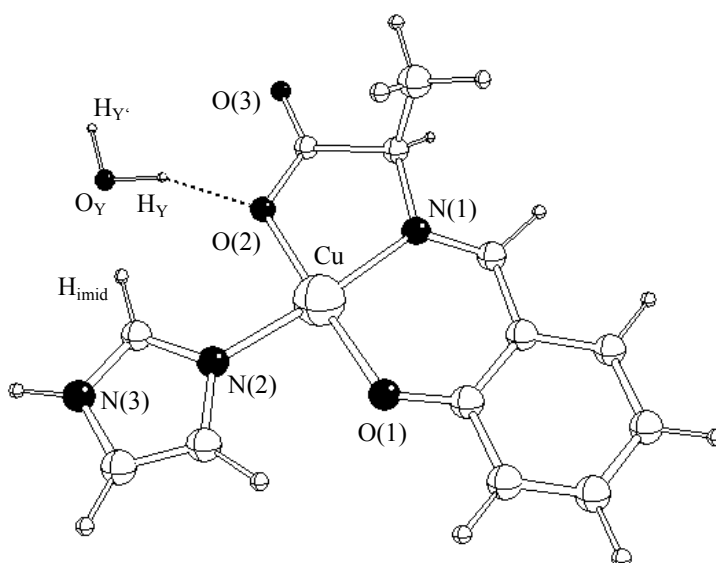


Fig. 4 Optimized geometry of CuSalala.imid...H₂O adduct hydrated at O(2) site (model C2)

No substantial CuSalala.imid geometry improvement of (Breza 2006a, 2006b) data due to diffusion functions inclusion related to the experimental structure A_{exp} (Warda 1998) may be concluded (Table 2). The differences may be explained by the dimer character of this X-ray structure. Unlike model B with substantially rebuilt Cu coordination sphere, its

geometry in C1 and C2 models exhibits only small differences. The Cu-O_Y bond in model B is substantially shorter than other Cu-O bonds whereas the remaining Cu-O/N bonds are longer than in the model A. In C1 and C2 models the less significant elongation of Cu-O_X bonds (i.e. at the addition sites only) may be observed. Whereas O_X...H_Y distances are shorter in C1 model than in C2, the reverse relation holds for the O_X...H_{imid} ones. The hydrogen bonds formation is reflected in the non-equivalence of O_Y-H_Y bonds in the adducts with water. Nevertheless, the O_Y...H_{imid} distance in B model is substantially shorter than the analogous ones in C1 and C2 models. Finally it must be mentioned that except B model no substantial changes in copper coordination polyhedra have been observed.

Table 1. Total energy without (E_{tot}) and with ZPE correction ($E_{\text{tot}} + \text{ZPE}$) and Gibbs free energy at 298 K (G_{298}) of the systems and reactions under study.

		E_{tot} [Hartree]	$E_{\text{tot}} + \text{ZPE}$ [Hartree]	G_{298} [Hartree]
Compound	Model			
OH ⁻		-75.79707	-75.78881	-75.80508
H ₂ O		-76.42267	-76.40161	-76.41993
CuSalala.imid	A	-2533.57976	-2533.32929	-2533.37801
CuSalala.OH ⁻ ...imid	B	-2609.48492	-2609.22347	-2609.27888
CuSalala.imid...H ₂ O	C1 ^{a)}	-2610.01260	-2609.73772	-2609.79080
	C2 ^{b)}	-2610.02001	-2609.74520	-2609.79872
Reaction				
A + OH ⁻ → B		-0.10809	-0.10537	-0.09578
A + H ₂ O → C1		-0.01016	-0.00682	+0.00714
A + H ₂ O → C2		-0.01757	-0.01430	-0.00078

^{a)} hydrated at O(1) site

^{b)} hydrated at O(2) site

Table 2. Selected geometrical parameters of the systems under study.

Model	X-ray data (Warda 1998)		DFT data		
	A _{exp}	A	B	C1	C2
Solvated site X	-	-	Cu	O(1)	O(2)
Total charge	0	0	-1	0	0
Y	-	-	OH ⁻	H ₂ O	H ₂ O
Bond lengths [Å]					
Cu – O(1)	1.930(2)	1.919	1.974	1.938	1.920
Cu – O(2)	1.965(2)	1.939	1.983	1.940	1.955
Cu – N(1)	1.933(3)	1.941	1.969	1.951	1.947
Cu – N(2)	1.966(3)	2.004	5.903	2.014	2.005
O _X – H _Y	-	-	-	1.838	1.926
Cu – O _Y	-	-	1.875	4.167	4.451
O _Y – H _Y	-	-	0.970	0.984 ^b /0.968	0.982 ^b /0.969
O _Y ...H _{imid}	-	-	1.717	2.333	2.102
Bond angles [°]					
O(1)-Cu-O(2)	176.25(10)	176.4	170.1	176.5	173.3
O(1)-Cu-N(1)	93.12(11)	94.1	91.6	92.7	93.6
O(1)-Cu-N(2)	92.37(11)	91.4	134.6	94.4	90.7
O(1)-Cu-O _Y	-	-	91.8	35.0	154.9
O(2)-Cu-N(1)	83.16(11)	84.5	83.1	83.8	83.6
O(2)-Cu-N(2)	92.37(11)	90.1	47.3	89.1	92.6
O(2)-Cu-O _Y	-	-	94.2	147.4	26.9
N(1)-Cu-N(2)	163.73(13)	173.9	128.4	172.2	174.1
N(1)-Cu-O _Y	-	-	174.4	118.7	110.5
Cu-O _Y -H _Y	-	-	106.0 ^a	23.6 ^b /105.1	34.2 ^b /137.7
O _X -H _Y -O _Y	-	-	-	168.9	155.3
Cu-O _X -H _Y	-	-	-	121.1 ^b /116.1	143.0 ^b /149.0
H _Y -O _Y -H _Y '	-	-	-	106.3	104.8
Dihedral angles					
O(1)-Cu-O _Y -H _Y	-	-	-5.6	18.3 ^b /114.4	-157.2 ^b /-176.6
O(1)-Cu-O _Y -H _{imid}	-	-	-149.6	113.1	31.6
Cu-O _X -H _Y -O _Y	-	-	-	98.1	19.0
O _X -H _Y -O _Y -H _Y '	-	-	-	-170.3	157.8

^a) Cu-O_X-H_Y bond angle is 119.6°

^b) bonded to O_X

Table 3. Selected MPA electronic structure data of the systems under study.

Model	A	B	C1	C2
Solvated site X	-	Cu	O(1)	O(2)
Total charge	0	-1	0	0
Y	-	OH ⁻	H ₂ O	H ₂ O
Atomic charges				
Cu	0.887	0.795	0.889	0.910
O(1)	-0.688	-0.661	-0.731	-0.686
O(2)	-0.628	-0.583	-0.627	-0.650
N(1)	-0.580	-0.551	-0.569	-0.579
N(2)	-0.610	-0.434	-0.625	-0.629
O _Y	-	-0.814	-0.766	-0.776
H _Y	-	0.345	0.414 ^a /0.345	0.417 ^a /0.351
d electron population				
Cu	9.126	9.127	9.129	9.126
Atomic spin densities				
Cu	0.687	0.708	0.690	0.696
O(1)	0.075	0.060	0.066	0.080
O(2)	0.086	0.071	0.090	0.073
N(1)	0.088	0.054	0.089	0.084
N(2)	0.051	0.000	0.051	0.052
O _Y	-	0.095	0.001	0.001
Overlap populations				
Cu – O(1)	0.183	0.161	0.166	0.180
Cu – O(2)	0.164	0.161	0.162	0.154
Cu – N(1)	0.167	0.146	0.161	0.167
Cu – N(2)	0.145	0.000	0.153	0.156
Cu – O _Y	-	0.221	0.006	0.002
O _X – H _Y	-	-	0.045	0.024
O _Y – H _Y	-	0.197	0.209 ^a /0.237	0.218 ^a /0.239
O _Y – H _{imid}	-	0.099	0.031	0.055

^a) bonded to O_X

Non-physical polarization and diffusion functions may cause significant errors in MPA characteristics and so our MPA results (Table 3) are evaluated at B3LYP/6-31G level only. The replacement of imidazole by OH⁻ ligand (with the most negative O_Y charge) causes the increase of Cu electron density and its decrease on the remaining atoms bonded to Cu. The C2 model is more sensitive on water addition than C1, the significant increase of Cu-O_Y bond polarity may be observed (unlike B model). The non-equivalence of water H_Y charges may be concluded as well.

Table 4. Selected NBO electronic structure data of the systems under study.

Model	A	B	C1	C2
Solvated site X	-	Cu	O(1)	O(2)
Total charge	0	-1	0	0
Y	-	OH ⁻	H ₂ O	H ₂ O
Natural charge				
Cu	1.345	1.381	1.348	1.354
O(1)	-0.827	-0.802	-0.856	-0.823
O(2)	-0.854	-0.821	-0.862	-0.869
N(1)	-0.620	-0.615	-0.613	-0.624
N(2)	-0.634	-0.576	-0.643	-0.647
O _Y	-	-1.201	-1.002	-1.016
H _Y	-	0.472	0.500 ^a /0.480	0.509 ^a /0.491
d electron population				
Cu	9.328	9.303	9.330	9.329
Natural spin densities				
Cu	0.588	0.615	0.591	0.596
O(1)	0.103	0.083	0.090	0.111
O(2)	0.108	0.091	0.114	0.092
N(1)	0.122	0.077	0.123	0.118
N(2)	0.078	0.000	0.051	0.071
O _Y	-	0.027	0.000	0.000
NAO bond order				
Cu – O(1)	0.186	0.172	0.161	0.182
Cu – O(2)	0.185	0.177	0.174	0.163
Cu – N(1)	0.197	0.187	0.191	0.195
Cu – N(2)	0.161	0.000	0.168	0.164
Cu – O _Y	-	0.208	0.002	0.002
O _X – H _Y	-	-	0.067	0.051
O _Y – H _Y	-	0.604	0.588 ^a /0.594	0.579 ^a /0.594
O _Y – H _{imid}	-	0.120	0.014	0.020

^a) bonded to O_X

Copper d electron density is the same for all the systems under study and corresponds to Cu(II). Spin density is located prevailingly at Cu in all models. Its increase in B model may be observed despite lower Cu charge. Overlap populations data confirm the above mentioned trends in bond lengths (the strongest Cu-O_Y bond and Cu-O/N bonds weakening in B model, Cu-O_X bonds weakening and non-equivalence of O_Y-H_Y bonds in C1 and C2 models). The same holds for the hydrogen bonds as well.

NBO population analysis is not influenced by non-physical character of the functions involved in atomic basis sets used and our results (Table 4) confirmed the most MPA trends mentioned above. Unlike MPA, NBO predicts more positive Cu charge in B model and more polarized Cu-O_Y bonds in C1 and C2 models. Despite some differences in values, the reasonable agreement of the remaining trends may be observed.

Finally it may be concluded that OH⁻ anions bond preferentially to Cu site in CuSalala.imid and replace the imidazole ligand. Water molecules form hydrogen bonds with both the oxygen atoms (but not with nitrogens) of copper(II) coordination polyhedron and weaken the Cu-O_Y bonds. It may be expected that in this way activated CuSalala.imid molecule reacts with sugar and this reaction is concurrent with the above mentioned hydroxylation at Cu site. It is evident that very high pH values of real solutions are not desirable for CuSalala.imid stability. Alternatively, its active centre must be shielded by a sugar molecule. Further experimental as well as theoretical studies are necessary to explain the mechanism of the sugars oxidation catalyzed by N-salicylideneaminoacidato copper(II) complexes with imidazole derivatives in the presence of sodium hydroxide in methanol/water and acetonitrile/water solutions.

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