9th Pannonian International Symposium On Catalysis

8 – 12 September 2008

Štrbské pleso
High Tatras National Park
Slovakia

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Published by the Slovak University of Technology in Bratislava
In the Publisher house of the Slovak University of Technology in Bratislava in 2008
Impress: 100 copies
486 pages

ISBN 978-80-227-2923-9

BIOINSPIRED CATALYSTS: SYNTHESIS, CHARACTERISATION AND SOME APPLICATIONS

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Abstract

Our recent work concerning the synthesis, characterisation and some applications of bioinspired electron-transfer catalysts is reviewed in this contribution. The catalysts were various mono- or heterobimetallic complexes having either Cu(II) or Cu(II) and Zn(II) as central ions and amino acids, their derivatives or various N-containing organic molecules as ligands. Emphasis was based upon the solid support immobilised versions of these complexes. They were built or anchored onto various kinds of supports (silica gel, montmorillonite, Merrifield's resin) with different methods (adsorption, ion exchange, covalent grafting). The resulting materials were characterised by a variety of instrumental (FT-IR, Raman, EPR [electron paramagnetic resonance] and atomic absorption spectroscopies, thermogravimetry) as well as computational methods. Their superoxide dismutase, catecholase and catalase activities were tested and some of them were found to be promising candidates as durable electron-transfer catalysts being close to the efficiency of the mimicking enzymes.

Keywords: enzyme mimics; immobilisation; characterisation; electron-transfer catalysts.

Preliminaries and objectives of the project

It is a general aim of modern catalysis to develop substances that are able to act as highly active and, more importantly, highly selective catalysts throughout the field of chemical synthesis. In order to achieve this goal various approaches may be tried. One of the most promising ways is attempting to mimic Nature's most efficient catalysts, the enzymes [1]. Even though living organisms contain several thousand enzymes for basically all conceivable chemical reactions, simply applying them in the synthesis of, e.g., commodity or specialty chemicals has at least one major obstacle: they are very sensitive to the environment, they work in a narrow temperature and pressure ranges and in aqueous solution. These shortcomings may be eliminated (i) if the enzyme is immobilised over various supports by methods preserving the catalytic activity and selectivity of the support-free enzyme or (ii) knowing or determining the active site of the enzymes and immobilising only that one. Both approaches are pursued in the world and promising results emerge.

In our laboratory we have chosen the second method concentrating on mimicking electron-transfer enzymes, mainly superoxide dismutase (SOD), however, the catalase as well as catecholase activities of the synthesised materials were also probed. Mono- or heterobimetallic complexes were built or anchored onto supports *via*

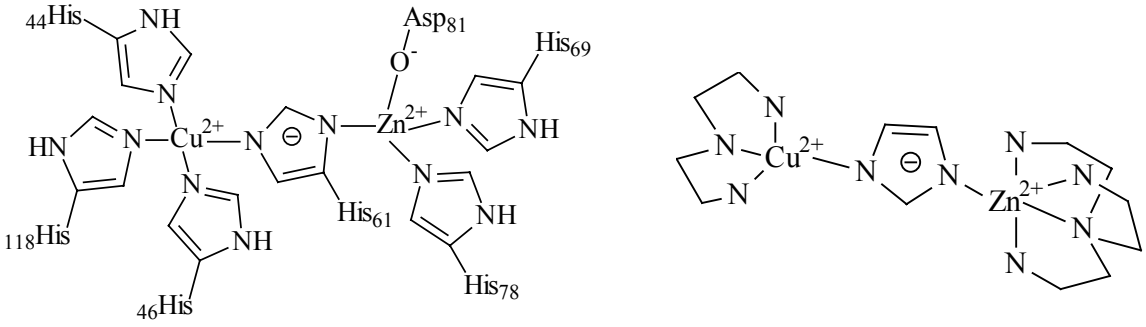
adsorption, ion exchange or covalent grafting. The supports were varied too, from the rigid silica gel, through montmorillonite giving confined environments for anchoring to Merrifield's resin (chloromethylated polystyrene) hoping to mimic the flexibility of the proteomic skeleton of the wild-type enzyme.

Comprehensive structural characterisation was attempted by all available instrumental methods and the SOD, catalase, catecholase and tyrosinase activities of the supported catalysts were probed by various test reactions. The SOD and the hydrolytic activities of the support-free heterobimetallic complex were also investigated.

Results of this still on-going project is reviewed in this contribution in chronological order, i.e. starting with the heterobimetallic Cu-Zn complex, bare and immobilised, followed by the monometallic Cu(II)-amino acid complexes, bare and immobilised.

The Cu-Zn complex bare and immobilised

The superoxide ion is harmful for living systems, thus, they need and have defence: they are able to eliminate the superoxide radical ion or at least decrease its concentration level through a dismutation reaction catalysed by enzymes called superoxide dismutases (SODs). Actually, these enzymes are of two main types, the manganese and iron SODs are found in prokaryotes (Mn, Fe), mithocondria (Mn) and plants (Fe), the copper-zinc SODs are most frequent in eukaryotic cells and in this version a Cu(II)–Cu(I) cycle does the catalysis. The structure of the active site in Cu-Zn SOD is known (Figure 1a) and this prompted the idea of the active site mimic, called Cu–Zn complex in the followings, displayed in Figure 1b.



(a) (b)

Fig.1. The active site of the superoxide dismutase (SOD) enzyme (a) and the active site mimicking Cu(II)-diethylenetriamino-μ-imidazolato-Zn(II)-tris(aminoethyl)amine cation (b).

Properties of the unsupported Cu–Zn complex

The complex was synthesized by Japanese authors first [2], but we discovered, characterised and exploited its SOD mimicking properties [3, 4] and after immobilisation we extended its application towards other electron transfer reactions [5] and phosphate ester hydrolysis [6].

It turned out that the aqueous solution of the complex itself displayed appreciable SOD activity by the method of Beauchamp and Fridovich [7]. The solution equilibrium and the binding mode of the species in the five-component system containing two metal ions (copper(II) and zinc(II)) and three ligands (**A** = diethylenetriamine [dien], **B** = imidazole [Im], **C** = tris(2-aminoethyl)amine) [tren] were investigated by pH potentiometric titration, UV–visible spectrophotometry and EPR spectroscopic titration in aqueous solution in the 2–11 pH range [3]. An imidazolate-

bridged heterobinuclear complex ($ACuBH_{-1}ZnC$) was found to evolve above pH = 7 and was stable between pH 7 and 11. The existence of the $ACuBH_{-1}ZnC$ complex was proved by mass spectrometry (ESI-MS (electrospray ionization mass spectrometry) and MALDI (matrix-assisted laser desorption/ionization) techniques). Indeed, this complex was proved to be responsible for the SOD activity.

The complex also displayed catecholase activity tested by the oxidation of 3,5-di-*tert*-butyl catechol to 3,5-di-*tert*-butyl quinone [5]. However, it was found that it had no catalase activity tested by the decomposition reaction of H_2O_2. Nevertheless, the host-free Cu-tren complex performed well in this transformation.

This same complex proved to be active in the hydrolysis of a 5' cap analogue, diadenosinyl 5',5'-triphosphate (ApppA), and two dinucleoside monophosphates: adenylyl(3',5')adenosine (ApA) and uridylyl(3',5')uridine (UpU) [6]. The catalytic activity of the complex was quantified by pseudo-first-order rate constants obtained in the excess of the cleaving agent. The results showed that the complex and its deprotonated forms had phosphoesterase activity and with ApppA the metal complex promoted cleavage took place selectively within the triphosphate bridge.

Properties of the immobilised Cu–Zn complex

The electron transfer reactions were only tested using the immobilised Cu–Zn complex and its precursors. Various supports and methods were applied for immobilisation [4, 8, 9]. The supports were montmorillonite (Mont) or silica gel (SG), the methods were adsorption (hydrogen bonding [h]), ion exchange [i] or covalent anchoring [c]. The resulting materials were structurally characterised by atomic absorption, FT-IR and EPR measurements. As far the SOD activities of the substances are concerned, it soon turned that immobilisation increased catalytic activities in general, anchoring the ready-made Cu–Zn complex onto silica gel by hydrogen bonding enhanced it in particular. The resulting catalyst came the closest to the activity of the Cu–Zn SOD enzyme (Table 1).

Table 1
Data (IC_{50} values) measuring SOD activity of the Cu-Zn SOD enzyme, the host-free complex and the immobilised complexes

Materials	IC_{50} (μmol/dm^3)	Ref.
Cu–Zn complex	69.1	[4]
Cu-Zn complex hydrogen bonded onto silica gel	6.0	[4]
Cu-Zn complex ion-exchanged into montmorillonite	91.0	[4]
Cu-Zn complex covalently grafted onto silica gel	134.0	[9]
Cu-Zn SOD	0.4	[4]

The catecholase activities showed similar trend (Table 2), while the immobilised Cu–Zn complexes did not display catalase activities, irrespective to the mode of anchoring. However, upon immobilisation the Cu–tren precursor of the Cu–Zn complex H_2O_2 decomposition proceeded with the following initial rate order [5]:

Mont-i-Cu–tren > SG-c-Cu–tren > SG-h-Cu–tren

Table 2
Initial rates in the oxidation reaction of 4,5-di-*tert*-butyl catechol promoted by the host-free Cu–Zn complex and the immobilised complexes

Materials	$r_o \times 10^5$ (mmol dm^{-3} min^{-1})	Ref.
Cu–Zn complex	32.9	[5]
Cu-Zn complex hydrogen bonded onto silica gel	1506.7	[5]
Cu-Zn complex ion-exchanged into montmorillonite	808.6	[5]
Cu-Zn complex covalently grafted onto silica gel	234.8	[5]

From SOD activity data it is seen that our Cu–Zn complex is a good SOD enzyme mimic and it is even better when it is immobilised. The activity is the highest upon anchoring the complex through hydrogen bonding. As EPR spectra also attest here, in a portion of the anchored complexes the geometry around the Cu ion becomes distorted tetrahedron from square planar, thus, the structure becomes strained to an extent, which is just appropriate for the dismutation reaction to occur with high efficiency. This distortion is also favourable for displaying high cathecolase activity. The geometry around the Cu ion resembles to that found in the SOD enzyme.

Immobilised Cu(II)–amino acid complexes

Cu(II)–amino acid complexes are quite often occurring active sites in various enzymes, mostly related to electron-transfer processes. The copper ion is a cofactor and the surrounding amino acids are part of the proteomic framework. The amino acids are of various types, but by far the most frequent is L-histidine. Histidine offers many coordination sites (imidazole ring nitrogens, amino nitrogen, carbonyl oxygen or deprotonated carboxylate oxygens). At the same time the ligand is able to participate in long-range proton transfer processes. Although for best approaching the real thing the active sites of copper-containing enzymes should be modelled by copper complexes with mixed amino acid ligands, to simplify the situation we started with the preparation of complexes with uniform amino acids. For more realistic modelling we at once went for building the complexes anchored to supports either to the more rigid silica gel, montmorillonite or the more flexible Merrifield's resin (with this latter we hoped to model the flexibility of the proteomic skeleton). After some initial attempts when immobilisation through hydrogen bonding onto silica gel [10, 11] or ion exchange into montmorillonite [12] was attempted, in order to have better control over the synthesis, we ended up with the covalent grafting procedure [13, 14]. The Merrifield's resin (chloromethylated polystyrene) and the chloropropylated version of silica gel were suitable supports for this and N- or C-protected amino acids were suitable reactants for further modification in a controlled manner. After or without deprotecting the amino acids the surface complex could be built by soaking the solid material in copper-containing solution with or without added amino acid. This way a large variety of surface anchored Cu(II)–amino acid could be prepared and this substance in spite of being uniformly ligated showed various enzymatic functionalities like SOD [9, 15, 16], catalase [17, 18] and tyrosinase[17, 18] activities.

The main objective of this part of the project was the preparation of covalently anchored copper–histidine complexes with total control of the synthesis and with enzymatic activity [16]. Applying protective groups ensured the control, however, it turned out that they have beneficial effects on, e.g., the SOD activity as well. Although anchored deprotected complexes showed SOD activities, surface complexes having protected amino acids as ligands displayed far better performance (Table 3). In this

respect there was no significant alteration whether N- or C-protected histidine was applied during the construction of the anchored complex.

Table 3
Data (IC_{50} values) measuring SOD activity of the Cu-Zn SOD enzyme and various host-free and anchored Cu(II)–histidine derivative complexes

Materials	IC_{50} (μmol/dm^3)	Ref.
Cu(II)–H-His-OH	108.6	[12]
Cu(II)–H-His-OH–montmorillonite	251.4	[12]
Cu,Zn SOD	0.4	[4]
BOC-His-OH–Cu(II)–BOC-His-OCH_2Phe-PS	13.4	[16]
PS-CH_2-His-OMe–Cu(II)–His-OMe	10.4	[16]
PS-CH_2-H-His-OH–Cu(II)–H-His-OH	199.4	[16]
H-His-OH–Cu(II)$^{-[BOC]}$H-His-OCH_2Phe-PS	61.5	[16]
PS-CH_2-H-His-OH$^{[OMe]}$–Cu(II)–H-His-OH	127.7	[16]

His – L-histidine; PS – polystyrene; BOC – *tert*-butoxycarbonyl; OMe – methoxy; [OMe] – from the C-protected histidine; [BOC] – from the N-protected histidine

Let me note that the complex immobilised through ion exchange showed the smallest SOD activity.

Regarding the outstanding SOD activities of the covalently grafted complexes bearing protective groups, a similar explanation is offered, once again supported by EPR spectroscopy, as for the anchored heterobimetallic complexes. The protected amino acids are more demanding sterically than the unprotected ones, therefore, they exert steric strain in the covalently attached complexes, just enough to induce the high activity. EPR data indicate distorted tetrahedral arrangement around the central copper ion. AAS measurements, FT-IR and Raman spectra point at 1:4 copper ion to protected histidine ratio having one imidazole nitrogen coordination per ligand.

Keeping in mind the previously discussed results, covalently grafted Cu(II)–N- or C-protected tyrosine complexes were prepared using chloropropylated silica gel or the Merrifield resin as support. During synthesis the solvent as well as the availability of ligands were varied [15]. There were experiments in which surface-anchored protected tyrosine molecules were only available (ligand-poor conditions), while in others, excess protected amino acid was also added (ligand-excess conditions). Isopropanol proved to be the most appropriate solvent and both anchored complex types displayed SOD activity albeit worse than with histidine derivatives as ligands (Table 4).

Table 4
Superoxide dismutase activities of the various surface-grafted copper–tyrosine derivative using silica gel as the support (Tyr – tyrosine; OMe – methoxy; BOC – *tert*-butoxycarbonyl; isopropanol as the solvent)

Silica gel supported Cu–amino acid complexes	IC_{50} (μmol/dm^3)
Ligand-poor conditions, H-Tyr-OMe ligand	208.9
Ligand-excess conditions, H-Tyr-OMe ligand	59.6
Ligand-poor conditions, BOC-Tyr-OH ligand	513.2
Ligand-excess conditions, BOC-Tyr-OH ligand	187.1

Data reveal that the structures of the surface complexes largely differ depending on the availability of ligands. Rational reasoning and the analysis of the FT-IR spectra

indicate that the tyrosine derivative acts as bidentate ligand under ligand-poor conditions, while two surface bound tyrosine derivatives and two added amino acid molecules are coordinated to the Cu(II) ion under ligand-excess conditions.

Future work

Presently and in the near future we are extending or activity in this area by varying the central ions [Co (II), Ni(II) and Fe(III)], the amino acids (cisteine and cystine derivatives) as well as the test reactions. In the not too distant future we hope that we can use some of these complexes in the synthesis of various commodity and specialty chemicals successfully.

Acknowledgement. This work was supported by the National Science Fund of Hungary through grant K62288. The financial help is highly appreciated.

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