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SOD ACTIVITY OF IMMOBILIZED ENZYME MIMICKING COMPLEXES

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Keywords Cu-Zn superoxide dismutase, Enzyme mimicking complex, Riboflavin/NBT assay, Immobilization Silica gel, Montmorillonite

Abstract

A binuclear, imidazolato-bridged, possible superoxide dismutase-mimicking complex (Cu(II)-diethylenetriamino-μ-imidazolato-Zn(II)-tris-aminoethylamine-triperchlorate) was prepared and immobilized on silica gel or among the layers of montmorillonite. The superoxide dismutase (SOD) activity of the complex before and after immobilization was studied by a SOD assay. It was found that the SOD activity of the host-free complex decreased somewhat when montmorillonite was the host, however, using silica gel as host it increased.

I. Introduction

A number of biological reactions in aerobic organisms have been proposed to involve the generation of superoxide anion. The superoxide radical ion is hazardous to living matter. There are indications that it reacts with the thiol and other groups of proteins [1]. Living systems have defense, however, 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, they 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 mostly in eukaryotic cells and in this version a Cu(II)–Cu(I) cycle does the catalysis. The reactions are as follows:

$$O_2^- \rightarrow O_2 + e^-$$

$$O_2^- + e^- + 2\ H^+ \rightarrow H_2O_2$$

The active center of superoxide dismutase (SOD) enzyme is known, it consists of copper(II) and zinc(II) ions bridged by a histidyl imidazolate anion [2]. The structure of Cu-Zn SOD is seen in the Figure 1.

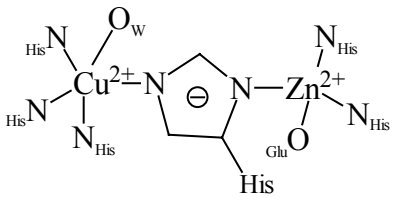

Figure 1. Structure of the active center of Cu-Zn SOD.

Generally speaking enzymes are very active and selective, however, quite sensitive catalysts. Changes in temperature, solvating properties, etc. may easily lead to denaturation (frequently irreversibly) that means the end of their catalytic activities. It would certainly be nice to have in our hands materials so active and selective as the enzymes but less sensitive than they are.

Several superoxide dismutase-mimicking complexes have been prepared investigated by various methods previously [3,10,11]. They were either mononuclear copper(II)- or binuclear copper(II)- and zinc(II)-containing models. These models had SOD activity, however, they were by far less efficient, but often less sensitive to reaction variables than the real enzyme. There is way of

further decreasing sensitivity. It is the immobilization of complexes in/on solid or semisolid matrices [4-6]. When it is done enzyme mimics may be obtained, which might be efficient and selective catalysts in a large variety of reactions.

During the work leading to this contribution we have prepared a Cu-Zn binuclear complex [7] hoping that it would effectively mimic the active centre of Cu-Zn SOD. The complex and its silica-anchored derivative were investigated by computational method earlier [8]. To increase stability the complex has been immobilized either on silica gel or in montmorillonite. The resulting materials were characterized structurally as well as their SOD activities were tested by riboflavin/NBT assay [9]. Results of these experiments are communicated in the followings.

II. Results

The structure of the binuclear host-free Cu-Zn complex (Cu(II)-diethylenetriamino-μ-imidazolato-zinc(II)-tris-aminoethylamine perchlorate) is seen in the Figure 2 [7].

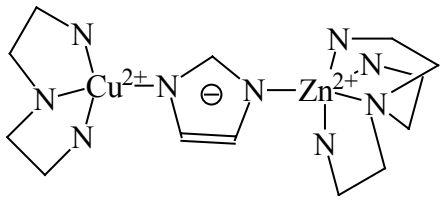

Figure 2. Structure of the Cu-Zn complex.

The complex itself shown considerable activity and fair stability in the decomposition of H_2O_2 [4-5] (*catalase* activity). It was hoped that solid enzyme mimics could be prepared, being stable and having at least as high SOD activity as the host-free complex.

The superoxide dismutase-like activity of the materials has been measured by the method of Beauchamp and Fridovich (the riboflavin/NBT) [9]. It is an indirect method, the superoxide anion radicals produced

on illumination by riboflavin, reacts with our complex, thus, inhibiting the reduction of NBT, i.e. the development of its blue color. In order to determine the concentration of Cu-Zn complex required yielding 50% inhibition of the reaction, the percentage of inhibition against copper(II) concentration was plotted. The value corresponding to IC_{50} of the host-free complex was 69.1 μmol/dm^3. Several SOD mimicking complexes have been studied lately by several methods [10-11]. The Cu-Zn complex has similar activity as the other copper(II) complexes, however, it is significantly less active than the native Cu-Zn SOD. Nevertheless, it is a potent SOD mimic considering its very low molecular weight compared to that of the native enzyme.

In our further work we used supports of increasing complexity. First, silica gel was applied, which may be taken as a first approximation planar (the pores are too wide to cause steric constraint), while montmorillonite by virtue of the layered structure provides a more closed system.

Adsorbing the complex on its surface performed immobilization on silica gel and washing off the portion, which was only weakly, attached. Immobilization took place *via* hydrogen bonding taking advantage of OH groups abundant on the silica gel surface. The oxygen of the OH groups could play the role of a hydrogen acceptor, while there were both hydrogen donor (carbon) as well as acceptor (nitrogen) atoms in the complex. Even though only a minute amount of complex could be attached to the surface of the silica gel (it could not be detected by FT-IR spectroscopy) surface modification was successful since after dissolving the solid material 15.8930 μmol/g (1010 ppm) copper(II) and the 15.66 μmol/g (1024 ppm) zinc(II) were measured by atomic absorption spectroscopy. The appearance of SOD activity indicates that the complex did not fall apart on adsorption. The SOD activity did not merely appear but it increased with an order of magnitude

compared to that of the host-free complex. The inhibition curve is displayed in Figure 3 and the IC_{50} value was found to be 6.0 μmol/dm^3.

In further experiments the silica gel support was replaced with montmorillonite. Montmorillonite is a layered material with cation exchange ability. It is capable of swelling, thus, the interlayer volume increases tremendously in a large variety of solvents, such as water and different alcohols. The complex was immobilized in this support exploiting its cation exchange and swelling capabilities. Again the excess complex was washed off during the aftertreatments. In this composite the amount of the immobilized complex was high enough to be detectable by IR spectroscopy. The immobilized material displayed SOD activity similarly to the others, except its IC_{50} value was 91 μmol/dm^3, even higher than that of the host-free complex (see in Figure 3).

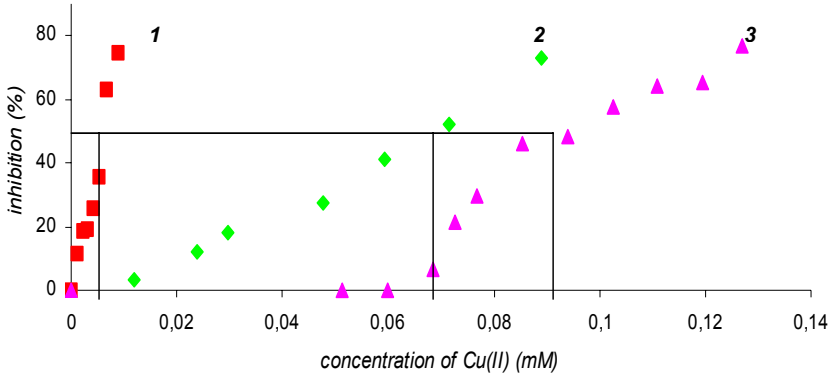

*Figure 3. Inhibition of NBT reduction by superoxide anion radical vs. the concentration of Cu-Zn complex (**1**) and of the Cu-Zn complex immobilized on silica gel (**2**) and Cu-Zen complex immobilized in montmorillonite (**3**). IC_{50} values are indicated on the concentration axis.*

Quite probably immobilization through electrostatic forces decreased the flexibility of the complex and the host having a less open system than silica gel may have decreased the availability of the prosthetic group *via* a stricter control of molecular traffic.

In order to facilitate an easier comparison the IC_{50} values measured by us and that of the native complex is summarized in Table 1.

Table 1. The SOD-like activity described by IC_{50} values.

Materials	IC_{50} (μmol/dm^3)	References
Cu-Zn complex	69.1	This work.
Cu-Zn complex immobilized on silica gel	6.0	This work.
Cu-Zn complex immobilized in montmorillonite	91.0	This work.
Cu-Zn SOD	0.004	[10]

III. Conclusions

Results described above and the data displayed in Table 1 make it clear that our complex is a good superoxide dismutase mimic. Upon immobilization the SOD activity retained and even if it was not increased (immobilization in montmorillonite) the obtained heterogeneous catalysis allowed easier workup and recycling than the host-free complex. The major result is, however, the finding that when the complex was anchored *via* hydrogen bonds to silica gel (a relatively open surface) enough mobility of the prosthetic group was preserved to provide even superior activity than that of the host-free complex and at the same time advantages of a heterogeneous system to the homogeneous one (easy workup and recyclability) are retained. Even though the native complex is still more active, but the activity of the silica gel anchored substance is a good approach and this material is certainly a more durable catalyst than the enzyme.

Acknowledgement

This work supported by the National Science Fund of Hungary through grant OTKA T034793. The financial support is highly appreciated.

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