Report on the project PD128326

"Investigations of equilibrium-, redox- and photoinduced reactions of metal-ligand systems inspired by nature"

1. Introduction

The main goal of this project was to gain insight into the coordination mode and redox processes of transition metal complexes modelling the active site of some oxidoreductase enzymes. Therefore, several new peptides and low molecular weight ligands were synthesized and investigated with transition metal ions.

In agreement with the work plan, an accurate description of the complex formation processes of NiSOD binding loop was achieved and the fine-tuning effect of the coordinated side chains was understood via the alternation of the side chains involved in nickel binding. These results were further corroborated by theoretical (Density Functional Theory and Molecular Dynamics) studies. New theoretical method was designed to calculate the electronic absorption spectra of copper(II) complexes. New copper(II) complexes bearing pyridine-2,6-carboxamide platform were synthesized and completely characterized, novel sulfosalan ligands and their transition metal (copper(II), nickel(II) and palladium(II) complexes were synthesized and their applications in C-C bond formation reactions were studied. An ultimate challenge of this project was to find a new experimental protocol to study the SOD activity of transition metal complexes. Herein, we designed a dedicated stopped-flow experiments with the corresponding data analysis. This experimental protocol was used to study the SOD activity of novel copper(II), nickel(II) and manganese(II) complexes. The results acquired during the project are discussed in the subsequent part of the report.

2. Methodology

The peptides were synthesized by solid phase peptide synthesis or purchased from commercially available sources. Low molecular weight ligands were synthesized according to the methods published in the literature. Peptides and ligands used in the project were characterized by several spectroscopic methods (^{1}H and ^{13}C NMR and mass spectrometry) and their purities were checked via pH-potentiometric titrations.

The stoichiometry and stability of metal complexes were determined by pH-potentiometry and/or UV-vis spectroscopy. The coordination mode of the complexes was characterized by UV-vis, circular dichroism, EPR, NMR and mass spectrometry. These results were further

corroborated by theoretical methods. Herein, we designed a new theoretical protocol to calculate the electronic absorption spectra of copper (II) complexes (see below).

3. Results

3.1 Thermodynamic, spectroscopic and kinetic features of NiSOD binding loop and its related catalytically active fragments

Detailed equilibrium, spectroscopic and SOD activity studies were reported on nickel(II) complexes formed with the *N*-terminally free HHDLPCGVY-NH_2 (**NiSODHH**) and HCDLPHGVY-NH_2 (**NiSODHC**) peptides mimicking the nickel binding loop in NiSOD. In these model peptides, cysteine was incorporated in different positions in order to gain better insight into the role of the cysteine residues in NiSOD. The results were compared with those obtained with the wild-type fragment of NiSOD. Our results confirmed, that the complex formation equilibria of nickel(II) with the two peptides exhibit different features. In the case of **NiSODHH**, the ligand field of the (NH_2,N_{Im},N_{Im},S^-) donor set is not strong enough to cause spin pairing and an octahedral paramagnetic complex is formed under physiological conditions. In contrast, **NiSODHC** forms square-planar diamagnetic complex with (NH_2,N^-,S^-,N_{Im}) donors which exhibits remarkable SOD activity. Our results unambiguously proved that the presence of cysteine in the secondary position of the peptide chain is crucial to establish the square-planar geometry in the reduced form of NiSOD, while the distant cysteine affects the redox properties of the Ni(II)/Ni(III) couple. Compared to the model systems, the Ni(II) complex with the wild-type fragment of NiSOD exhibits superior SOD activity. This confirms that both cysteinyl residues are essential in efficient degradation of superoxide ion. The enzyme mimetic complexes are also capable to assist the decomposition of superoxide ion, however, they show considerably smaller catalytic activity due to the absence of one of the cysteine residues.

We also studied a nickel complex formed with the metallopeptide bearing two nickel binding loops of NiSOD. The metallopeptide exhibits unique nickel binding ability and the binuclear complex is a major species with 2x(NH_2,N_{amide},S^-,S^-) donor set even in an equimolar solution of the metal ion and the ligand. Nickel(III) species were generated by oxidizing the Ni(II) complexes with KO_2 and the coordination modes were identified by EPR spectroscopy. The binuclear complex formed with the binding motifs exhibits superior SOD activity, in this respect it is an excellent model of the native NiSOD enzyme. A detailed kinetic model is postulated which incorporates spontaneous decomposition of the superoxide ion, the dismutation cycle and fast redox degradation of the binuclear complex. The latter process leads to the elimination of the SOD activity. A unique feature of this system is that the Ni(III) form

of the catalyst rapidly accumulates in the dismutation cycle and simultaneously the Ni(II) form becomes a minor species.

Multidisciplinary protein design was also reported on a metallopeptide mimicking the binding loop of nickel containing SOD enzyme. D-penicillamine, a natural decomposition product of penicillin, was introduced into the peptide chain yielding the H(Pen)DLPCGLY (**wtPen**) peptide. The nickel(II) binding ability of **wtPen** was characterized by thermodynamic, spectroscopic and computational techniques (full DFT and Molecular Dynamics methods). Oxidation of the Ni(II) complex by KO_2 yields a square-pyramidal Ni(III) species coordinated by the axial His-N in a well-defined α-helix folding state. The structure of the Ni(III) species was analyzed by EPR spectroscopy and theoretical methods confirming that the donor set involved in the metal ion coordination and the folding state are retained after oxidation. The complex exhibits superior SOD activity which was studied by sequential stopped-flow method. Thorough analysis of the data shows that the Ni(III) species rapidly accumulates in the nickel catalyzed decomposition of superoxide anion. Accordingly, the presence of the penicillamine moiety close to the catalytic center increases the life-time of the Ni(III) transient species. In contrast, Ni(III) exists only at relatively low concentration level in the dismutation reaction catalyzed by the native SOD enzyme fragment.

3.2 Investigation of the transition metal complexes of sulfonated salan ligands

With the aim of identifying new types of water-soluble catalyst precursors for modification of biological membranes by homogeneous hydrogenation in aqueous solution and under mild conditions, we have performed detailed equilibrium and spectroscopic characterization of complex formation between nickel(II) or palladium(II) and salan-type ligands sulfonated in their aromatic rings (**HSS**, **PrHSS** and **BuHSS**) in the slightly acidic – alkaline pH range. The stability constants of the metal complexes were determined using pH-potentiometry. The catalytic activities of the [Ni(**HSS**)] and [Pd(**HSS**)] complexes in hydrogenation and redox isomerization of oct-1-en-3-ol were also studied. The results indicate, that all of the investigated ligands exhibit excellent nickel(II) and palladium(II) binding ability via the formation of (O^-,N,N,O^-) linked chelate system. Both [Ni(**HSS**)] and [Pd(**HSS**)] catalyze the hydrogenation and redox isomerization of oct-1-en-3-ol. [Pd(**HSS**)] shows excellent activity and the reaction was highly selective to the formation of octan-3-ol. [Ni(**HSS**)] is also an active and selective catalyst for this hydrogenation reaction and to the best of our knowledge, [Ni(**HSS**)] is the first nickel(II)-based, hydrolytically stable, water-soluble catalyst bearing sulfonated salan moiety.

Copper(II) complexes formed with sulfonated salan ligands (**HSS**) have been synthesized and their coordination chemistry has been characterized using pH-potentiometry and spectroscopic methods (UV-Vis, EPR, EDNMR) in aqueous solution. Several bridging moieties between the two salicylamine functions were introduced e.g., ethyl (**HSS**), propyl (**PrHSS**), butyl (**BuHSS**), cyclohexyl (*cis*-**CyHSS**, *trans*-**CyHSS**) and diphenyl (**dPhHSS**). All the investigated ligands feature excellent copper(II) binding ability *via* the formation of (O^-,N,N,O^-) chelate system. The results indicated that the cyclohexyl moiety significantly enhances the stability of the copper(II) complexes. EPR studies revealed that the arrangement of the coordinated donor atoms is more symmetrical around the copper(II) center and similar for **HSS**, **BuHSS, CyHSS** and **dPhHSS**, respectively, and higher rhombicity of the ***g*** tensor was detected for **PrHSS**. The copper(II) complexes of the sulfosalan ligands were isolated in solid form, too, and showed moderate catalytic activity in the Henry (nitroaldol) reaction of aldehydes and nitromethane. The best yield for nitroaldol production was obtained for copper(II) complexes of **PrHSS** and **BuHSS**, although their metal binding ability is moderate compared to the cyclohexyl counterparts. However, these complexes possess larger spin density on the nitrogen nuclei than for the other cases, that alter their catalytic activity.

3.3. DFT studies on copper(II)-bioligand systems

The visible region of the electronic absorption spectra of Cu(II) complexes was studied by TD-DFT methods. The performance of twelve functionals in the prediction of λmax was tested on eleven compounds with different geometry, donors and charge. The ranking of the functionals for λmax was determined in terms of MAPD (mean absolute percent deviation) and SD (standard deviation) and it is as follows: BHandHLYP > M06 >> CAM-B3LYP >> MPW1PW91 ~ B1LYP ~ BLYP > HSE06 ~ B3LYP > B3P86 ~ ω-B97x-D >> TPSSh >> M06-2X (MAPD) and BHandHLYP > M06 ~ HSE06 > ω-B97x-D ~ CAM-B3LYP ~ MPW1PW91 > B1LYP ~ B3LYP > B3P86 > BLYP >> TPSSh >> M06-2X (SD). With BHandHLYP functional the MAPD is 3.1% and SD 2.3%, while with M06 the MAPD is 3.7% and SD is 3.7%. The protocol validated in the first step of the study was applied to: i) calculate the number of transitions in the spectra and relate to the geometry of Cu(II) species; ii) determine the coordination of axial water(s); and iii) predict the electronic spectra of the systems Cu(II)–human serum albumin and Cu(II) bound to the regions 94-97 and 108-112 of prion protein. The results indicate that the ì proposed computational protocol allows a successful prediction of the electronic spectra of Cu(II) complexes and to relate an experimental spectrum to a specific structure.

This experimental protocol was also used to study novel Sb(III) complex. In this work we reported a detailed coordination chemistry study on [Sb(**PCTA**)] (**PCTA**: 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid), a macrocyclic aminopolycarboxylate type complex of antimony(III), whose ^{119}Sb isotope could be a suitable low-energy electron emitter for radiotherapy. The thermodynamic stability of the chelate obtained by pH-potentiometry and UV-vis spectrophotometry is high enough to prevent the hydrolysis of metal ion near physiological pH. The formation of [Sb(**PCTA**)] is confirmed by NMR and ESI-MS measurements in solution, furthermore the structure of [Sb(**PCTA**)]·NaCl·3H2O and [Sb(**PCTA**)]·HCl·3H2O is described by X-ray diffraction and DFT calculations. Consequently, the [Sb(**PCTA**)] is the first, thermodynamically stable antimony(III) complex bearing polyamino-polycarboxylate macrocyclic platform. Our results demonstrate the potential of rigid (pyclen derivative) ligands as chelators for future application of Sb(III) in the targeted radiotherapy based on ^{119}Sb isotope.

3.4 Synthesis and characterization of novel SOD mimics

New copper(II) complexes of pyridine based ligands functionalized with alanine (**PydiAla**) and tyrosine (**PydiTyr**) moieties have been synthesized and characterized by pH-potentiometric, spectroscopic (UV-vis, CD, MS, EPR), computational (DFT) and X-Ray diffraction methods. Both ligands form high stability copper(II) complexes *via* the (N_{py},N^-,N^-) donor set supported by the binding of the carboxylate pendant arms. Although the coordination mode is the same for the two systems, the tyrosine containing counterpart exhibits increased copper(II) binding affinity, which is most likely due to the presence of aromatic moiety of the side chains. Both copper(II) complexes exhibit superior SOD activity, IC_{50} = 354 ± 10 nM and 28 ± 7 nM for the **PydiAla** and **PydiTyr** complexes, respectively. The about one order of magnitude higher activity of the **PydiTyr** complex is probably due to the presence of phenolic-OH group, which promotes the binding of the superoxide anion radical to the metal center. The results serve as a basis for designing highly efficient copper(II) mimics for medical and practical applications.

N-oxides of N-heteroaromatic compounds were also used in this part of the project. Herein, we provided a straightforward method for the synthesis of a series of mono-*N*-oxides of 1,10-phenanthrolines. The parent compounds were oxidized by a green oxidant, peroxomonosulfate ion in acidic aqueous solution. The products were obtained in high quality and at good to excellent yields. A systematic study reveals a clear-cut correlation between the basicity of the compounds and the electronic effects of the substituents on the aromatic ring. Complex

formation processes of the ligands with transition metal ions are still in progress in our laboratory.

3.5 Designing of new method to study SOD activity

In order to gain insight into the superoxide disproportionation mechanism, we designed new experimental protocol to study the SOD activity of superoxide dismutase mimics. In this direct method, we utilized the stopped-flow technique which rapidly mixes the stock solution of superoxide anion with the putative SOD mimics and the absorption of the superoxide can be followed by spectrophotometry. Unlike pulse radiolysis, the initial concentration of superoxide can be high compared to the SOD mimics in the stopped-flow experiments and the dismutation reactions can directly be monitored under real catalytic conditions. In the simplest case, first-order decay of superoxide was obtained after mixing the solutions of KO_2 and the buffer containing the SOD mimics, and the fundamental features of the catalysis (effects of catalyst concentration, pH, temperature, ionic strength) can easily be investigated. Consequently, this method allows the direct determination of the catalytic rate constant (k_{cat}) under a given set of conditions. However, such a simple kinetic feature diminishes in the case of mimics with relatively low catalytic activity – $k_{cat} < 10^{5.5}$ $M^{-1}s^{-1}$ (at pH = 7.4) – due to the competing second-order self-dismutation of the superoxide ion.

For the NiSOD related metallopeptides, kinetic traces cannot be fitted with such a simple expression (k_{cat}). After the fast initial phase, the rate of the absorbance change slowly decreases, i.e. the rate of $O_2^{-\bullet}$ decomposition declines and reaches the non-catalytic limit at longer reaction time. This feature is due to the kinetic coupling of the dismutation steps with the redox degradation of the catalyst.

This new method was also used to study manganese(II) complexes. We reported the Mn(II) complexes with two pyclen-based ligands (pyclen = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene) functionalized with acetate pendant arms either at positions 3,6 (**3,6-PC2A**) or 3,9 (**3,9-PC2A**) of the macrocyclic fragment. We confirmed, that the Mn(II) complexes of **3,6-PC2A** and **3,9-PC2A** are capable to assist the decomposition of superoxide anion radical. The kinetic rate constant of the complex of **3,9-PC2A** is smaller by one order of magnitude than that of **3,6-PC2A**.

4. Summary

The results of the project were published in 12 papers, and the PI was first and/or corresponding author in 8 publications. Moreover, 4 manuscripts are submitted to be considered for publication. Here, the PI is first and/or corresponding author. The results of the project were also demonstrated through presentations (2 posters and 14 oral contributions) in local and international conferences.

Debrecen, 19th of October 2021

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