EUROPEAN ORGANISATION FOR NUCLEAR RESEARCH

Status report for IS602

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Cu(I), Ag(I), Cd(II), Hg(II), and Pb(II) binding to biomolecules studied by Perturbed Angular Correlation of γ-rays (PAC) spectroscopy

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L. Hemmingsen[1], Peter W. Thulstrup[1], V. L. Pecoraro[2], A. Jancso[3], Jens Müller[4], K. Johnston[5], Monika Stachura[6], J.G.M. Correia[5]

[1]Department of Chemistry, University of Copenhagen, Denmark
[2]Department of Chemistry, University of Michigan, USA
[3]Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary
[4]Department of Inorganic and Analytical Chemistry, Westfälische Wilhelms-Universität Münster, Germany
[5]ISOLDE, CERN
[6]TRIUMF, Canada

Spokesman & author: L. Hemmingsen

Local contact: K. Johnston

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Abstract

Metal ions display diverse functions in biological systems and are essential components in both protein and nucleic acid structure and function, and in control of biochemical reaction paths and signalling. Similarly, metal ions may be used to control structure and function of synthetic biomolecules, and thus be a tool in the design of molecules with a desired function. In this project we address a variety of questions concerning both the function of metal ions in natural systems, in synthetic biomolecules, and the toxic effect of some metal ions. All projects involve other experimental techniques such as NMR, EXAFS, UV-Vis, fluorescence, and CD spectroscopies providing complementary data, as well as interpretation of the experimental data by quantum mechanical calculations of spectroscopic properties.

Summary of research progress and difficulties encountered within the IS602 projects

The main successes have been achieved using ^{199m}Hg PAC spectroscopy in combination with conventional spectroscopy and quantum calculations of spectroscopic properties on proteins and model systems:

1) The CueR metalloregulatory protein controls the intracellular concentration of Cu(I), and very selectively responds to Cu(I), Ag(I) and Au(I), but not to divalent metal ions. We have demonstrated that the metal site structure changes upon binding of Hg(II) by recruiting additional ligands from a C-terminal CCHH motif, and this may be a mechanism to achieve selectivity against divalent metal ions [Balogh et al., Chem. Eur. J., 2019, 25, 15030 – 15035].

   This project is still ongoing and the next aim is to explore protein variants where the C-terminal CCHH motif is deleted, as well as the effect of mutating the conserved Ser77 residue, which may have an effect on metal ion selectivity. Moreover, we have put emphasis on the opportunity to elucidate nanosecond dynamics at protein metal sites [Chakraborty et al., Acc. Chem. Res., **2017,** 50, 2225-2232], as well as the general application of PAC spectroscopy in chemistry and biochemistry [Jancso et al., J. Phys. G, **2017**, 44, 064003].

2) New projects have been initiated on:

   a. The BRI2 protein which has been demonstrated to affect aggregation of the Aβ protein and thus possibly be related to Alzheimer's disease. Collaboration with Marek Luckowski (University of Wroclaw). The main purpose of this project is to elucidate the BRI2 metal ion binding of e.g. Hg(II).

   b. So-called zinc-hook proteins where folding and stability is controlled by the presence of Zn(II), and we have explored the effect of Hg(II), most notable if this metal ion may also mediate formation of protein dimers and characterization of the metal site structure at various pH values. In collaboration with Artur Krezel and Marek Luckowski (University of Wroclaw)

   c. A model systems of the metalloregulatory protein (ArsR) which provides bacteria with resistance to arsenic in collaboration with A. Jancso (University of Szeged, Hungary). The main purpose of this project is to elucidate the metal site structure, and if it changes with experimental conditions such as protein to metal ion ratio.

   d. Proteins related to cataract of the eye (γ-crystallins), which are extremely sensitive to Hg(II) induced aggregation. Collaboration with Lilliana Quintanar, Centro de Investigación y de Estudios Avanzados (Cinvestav), Mexico City, Mexico. The main purpose ofthis project is to characterize the metal site structure of Hg(II)-protein complexes at different stages of the aggregation process.

The main difficulties have been have been encountered using the ^{68m}Cu and ^{111}Ag isotopes:

1) Due to problems extracting the implanted ^{111}Ag from the target (e.g. polyethylene) the ^{111}Ag PAC experiments at ISOLDE were discontinued. An alternative production of ^{111}Ag (neutron irradiation of ^{110}Pd at ILL, France) turned out to be possible, and has resulted in useful ^{111}Ag PAC experiments - although some optimization is still to be realized in the radiochemical separation of Pd and Ag.

2) In order to test the feasibility of using Cu isotopes in PAC studies, pilot experiments were conducted on ^{68m}Cu. The motivation is given by presence of Cu in a large number of proteins involved in electron transfer and activation of oxygen and lack of a suitable spectroscopic methods allowing for probing Cu(I). Unfortunately, it turned out that the electric quadrupole moment of the relevant nuclear level of ^{68m}Cu is too small to allow for straightforward use of this isotope in PAC experiments on proteins (as judged from experiments on Cu_2O). We have therefore terminated this subproject.

V. Experimental Methods

Several TDPAC spectrometers are available at ISOLDE, and will be used in the current projects. The synthesis and purification of the proteins will be done at the laboratory at the University of Michigan, and at the University of Szeged. The proposed experiments require the implantation for ~60 minutes into ice at 100 K (in order to guarantee the vacuum in the beamline). For this we use the so-called "biophysics chamber". We have developed our own target holder, cooled by liquid nitrogen, at the collection point. Occasionally, we subsequently add inactive carrier (metal ion salts dissolved in adequate buffer solutions) to the molten ice to increase the metal concentrations from nanomolar to micromolar. After the incubation of the biomolecules with the radioisotope containing-solution with or without carrier for 5 to 30 minutes the biomolecules are immobilized by precipitation/centrifugation, freezing, or adding sucrose. For the radiochemical preparation steps the fume hood of the Solid State Physics Laboratory in building 508 is required. There is also enough space for the cooling centrifuge, a refrigerator/freezer for protein samples, and other basic lab equipment, in the chemistry lab.

VI. Ion beams requested

Isotope	$T_{1/2}$	Target	Yield (ions/μC)	Ion source
^{111m}Cd	48 min	Sn	2×10^8	HP (VADIS)
^{199m}Hg	42 min	Pb	2×10^8	HP (VADIS)
^{204m}Pb	1.13h	UCx	2×10^8	RILIS (Pb)

Conclusion

We aim to put our focus on ^{199m}Hg, ^{111m}Cd and ^{204m}Pb PAC spectroscopy for the use of the remaining shifts of the IS602 proposal, completing the ongoing projects described above. Moreover, a number of projects on de novo designed proteins and metalloregulatory proteins in the original IS602 proposal are still to be completed or initiated (revised sections of the original IS602 proposal is attached below as an appendix).

Appendix: Revised sections of the original IS602 proposal related to projects still to be initiated or completed

Metal ion binding to de novo designed proteins

Over the past decade we have had considerable success exploring the biological chemistry of heavy metal ion binding to designed and naturally occurring proteins[3-8]. The synthetic proteins are excellent scaffolds to explore and design new metal ion binding sites, but also serve as model systems of naturally occurring regulatory proteins, metal chaperones and the targets of metal toxicity. The strength of this work has been directly dependent on the ability to carry out PAC studies of these systems, and PAC was the only technique to demonstrate that our original design for Cd(II) proteins was complicated by two species. Even metal site dynamics on the biologically important but elusive nanosecond time scale has been revealed[9].

We aim to continue this line of research, and our future studies will focus on 1) Achieving the first unambiguous ^{204m}Pb-PAC results for Pb(II) binding to a biomolecule. Initially we will conduct experiments on the synthetic homotrimeric proteins TRIL16C and TRIL19C, which despite presenting similar metal ion binding sites with three thiols, display very different chemical shifts for the Pb(II) ion in NMR spectroscopy. If successful these studies will be advanced further by experiments including synthetic heterotrimeric systems, from AAA, AAB, ABB, to BBB (where A and B are different monomers), to characterize the structural space of Pb(II) metal site coordination geometries, and thereby acquire basic knowledge about possible structures that Pb(II) may target in biological system, elucidating the toxicity of this metal ion. 2) Continuing to examine Hg(II) coordination geometry and metal site dynamics in HAH1[5], in order to assess how this toxic metal may be transported within human cells. Additionally, both binding of Hg(II) and Cd(II) to synthetic so-called A3D proteins resembling like rubredoxin and possible cupredoxins may shed light on heavy metal ion binding to these proteins families. Thus, the combination of different elements should allow us to make significant new advances in studying the basic chemistry of heavy metals in biology and further develop the field of de novo protein design.

Metal ion biosensors and model systems

It is believed that the survival and virulence of pathogenic bacteria inside mammalian hosts is largely related to the host metal-ion status and how bacteria respond to it, i.e. how they adapt to, exploit or influence the actual metal ion level[10]. In this bacterial response, the metal ion homeostasis and resistance systems play an important role. The elements of these systems are mediated by metalloregulatory proteins at a transcriptional level. These molecules are able to sense a specific metal ion or a small group of metal ions and this selectivity is, in part, related to the metal ion binding features of the proteins. The copper efflux regulator MerR family member CueR responds to the monovalent group 11 metal ions (Cu^I,Ag^I,Au^I) but shows no activity in the presence of the divalent ion Hg^{II} or Zn^{II}.[11] Crystal structures of CueR showed that M^I ions are restricted to a linear coordination environment by the two Cys-residues of the metal binding loop[11]. In the proposed project the intrinsic metal ion binding characteristics of the protein, i.e. the local structure of metal ions within the effector binding domain are planned to be investigated by means of PAC-spectroscopy. The utilization of various radionuclides (^{199m}Hg, ^{111m}Cd) may allow observing fundamental structural (geometry) changes promoted by the different type of metal ions which necessarily affect the conformation of the protein and thus its activity. Mutations within the metal binding loop of CueR and studying the metal ion binding of

the mutant proteins or relevant model peptides[12] may shed light on the role of individual residues in providing the optimal coordination "pocket" for the cognate metal ions.

Cadmium(II) is often applied as a substitute of zinc(II) ion in biomolecules for better understanding of the local structure of the metal ion and its function. Recently we investigated the metallonuclease colicin E7, the catalytic domain of which is a possible building block of artificial nucleases[13]. To apply it for this purpose we need to know the properties of this nuclease in detail. The metal ion binding, coordination environment and function was already studied and published in the literature, but the results are inconsistent and contradictory. Therefore, we would like to study the role and behaviour of the zinc(II) ion in the active centre in the presence and absence of the substrate. As the most common site-specific artificial nucleases consist of zinc finger DNA binding domains[14], the Zn(II) vs. Cd(II) substitution may also affect the mechanism of action and DNA recognition specificity. The precise description of the structural changes around the metal ion may be achieved by PAC spectroscopy.

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