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^{204m}Pb: A New Probe for TDPAC Experiments in Biology Complementing the Well Established Probes ^{111m}Cd and ^{199m}Hg

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Abstract

The short-lived nuclear probes ^{111m}Cd ($t_{1/2}$ = 49 min), ^{199m}Hg ($t_{1/2}$ = 43 min), and ^{204m}Pb ($t_{1/2}$ = 43 min) supplied by ISOLDE are used to study the interaction of metals with biological macromolecules like, e.g., DNA and proteins. The structure and dynamics of metal sites in biomolecules are important in determining the functional efficiency of these macromolecules. Many life processes are based on such interactions.
In order to study those metal sites close to physiological conditions a highly sensitive spectroscopic method is required, like Time Differential Perturbed Angular Correlation (TDPAC). Here, a radioactive atom is placed at the site of interest and by correlating the emitted γ-quanta in space and on a nanosecond time scale local structural information is provided via the Nuclear Quadrupole Interaction. These investigations will allow a deeper insight into the adaptivity and rigidity of metal sites in the blue copper proteins (electron transfer proteins), the binding and heavy metal detoxification processes with metallothioneines, switches, and also the development of heavy metal sensors based on DNA molecules.

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I. Introduction

Numerous chemical processes in the biosphere like transformations, transport phenomena and regulatory mechanisms are effected by metal containing molecules. Metal cores in such molecules, especially proteins, possess distinct ligation and coordination geometries with spectroscopic properties which are unusual compared to those of small molecular inorganic complexes of the same metal ion. In recent years more and more three dimensional structures of proteins have been determined by X-ray crystallography and for smaller molecules (molecular weights < 30 kD) also by multidimensional nuclear magnetic resonance (NMR). However, a lot of these macromolecules successfully evade crystallization and/or have molecular weights too large for NMR. Besides the well designed structure the structural flexibility is equally essential for their function. Due to this and the serious limitations of structural investigations in the field mentioned above, further information on structural and dynamic properties of metal sites is desired. Such information is supplied by spectroscopic techniques like UV/Vis absorption, "hetero-atomic" NMR, electron spin resonance (EPR/ESR), magnetic circular dichroism (MCD), X-ray absorption (XAS, EXAFS, XANES), Mössbauer effect and synchrotron-based Mössbauer effect (for references see [Tro99]). However, these techniques require large amounts of protein, i.e. unphysiological protein concentrations up to mM, and sometimes also temperatures down to 4 K. In contrast to these methods the use of suitable unstable nuclei as "nuclear probes" for Time Differential Perturbed Angular Correlations of γ-rays ($\gamma\gamma$ TDPAC) facilitates studies close to physiological conditions in the ultra-trace regime. Here, a radioactive atom is placed at the site of interest and by correlating the emitted γ-quanta in space and on a nanosecond time scale local structural information is provided. Mainly, the nuclear quadrupole interaction (NQI), i.e. the interaction between the electric field gradient, characterised by the nuclear precession frequency ω or the nuclear quadrupole coupling constant υ_Q and the asymmetry parameter η, is employed for this purpose. These investigations allow a deeper insight into the detoxification processes, genetic switches, adaptivity and rigidity of metal sites in biomolecules [Tro99].

II. Actual Status of Research

For biological studies mainly the isomeric TDPAC probes ^{111m}Cd ($t_{1/2}$ = 49 min) and ^{199m}Hg ($t_{1/2}$ = 43 min) are used because after-effects are negligible [Tro99,Tro01]. However, also the probe ^{111}Ag(β^-)^{111}Cd was successfully employed for this purpose whereas the working horse ^{111}In(EC)^{111}Cd of TDPAC spectroscopy in solid state physics proved to be practically useless. With ^{111m}Cd and ^{199m}Hg produced at ISOLDE we tackled so far the open questions of

- the metal coordination in the so-called “type 1” metal sites in blue copper proteins like azurin, stellacyanin or ascorbate oxidase and laccase [But97, But97a];
- the metal binding in rubredoxin [Fall00] and hemocyanin [Tro02b];
- the binding and reaction mechanism of Hg in detoxifcation proteins like the MerR protein or metallothioneines [Tro01a, Lei03];
- the fixation of heavy metals in soils and aquifers by humic substances [Kup96].

A survey of these topics is given in [Tro99,Tro01].

Besides the already mentioned isotopes ^{111m}Cd and ^{199m}Hg there is only one more TDPAC probe with an isomeric transition and convenient nuclear properties for PAC spectroscopy: ^{204m}Pb ($t_{1/2}$ = 67 min). Here, the 912 keV – 375 keV cascade with an effective anisotropy of 18 % is used. The intermediate state has a nuclear spin I = 4, a half-life of τ_N = 265(10) ns, a nuclear quadrupole moment of Q = 0.44(2) barn, and a magnetic dipole moment of μ = +0.225(4) μ_N. This probe was recently used for the determination of the electric field gradient in cadmium metal [Tro00]. It has to be emphasized that this isotope has an integer nuclear spin, whereas the ^{111m}Cd and ^{199m}Hg have half-integer spins (I = 5/2). For integer spins the eigenvalues of the NQI Hamiltonian operator for small asymmetry parameters η depend linearly on η whereas for half-integer spins quadratically. Hence, the ^{204m}Pb TDPAC probe is much more sensitive to deviations from axial symmetry than the two other isomeric TDPAC probes.

In an early effort in the seventies of the last century the nuclear quadrupole interaction of the isomeric TDPAC isotope ^{204m}Pb was investigated in a variety of metals and compounds [Haa73]. However, in some cases only a rough estimate of the NQIs in these compounds was possible due to the poor statistical quality of the data and serious limitations of the data analysis. In 2001 we could produce this isotope for the first time at the on-line isotope separator ISOLDE at CERN and determined the NQI of ^{204m}Pb in cadmium metal which was a factor of 2 different from a previous TDPAC experiment [Tro02a].

In 2002 we extended our investigations to Pb(II) complexes which were precipitated from aqueous solutions. In fig.1 the TDPAC spectra and their Fourier transforms from Pb(II) halides together with the spectra of Pb(II)-sulphate and Pb(II)-oxalate are shown.

The latter two Pb(II) complexes may also serve as model compounds for the structural investigations of biological macromolecules, the final goal of these investigations. Whereas the TDPAC-probes probes^{111m}Cd and ^{199m}Hg are predominantly used for studying proteins the ^{204m}Pb TDPAC probe can also be used for investigating nuclear acids, like DNA. Figure 2 displays the first ^{204m}Pb spectra of a Pb(II) binding catalytic DNA molecule together with the change in the fluorescence yield of the Pb-DNA complex. Due to their high sensitivity of fluorescence activity these biomolecules might serve as metal sensors. The first experiments with DNA are preliminary but promising. The fit of this spectrum is just to guide the eye.

Besides the experiment at the ISOLDE facility we performed also ^{204m}Pb experiments in our "home laboratory" at the University of Leipzig with the help of a ^{204}Bi ($t_{1/2}$=11.4 h)/^{204m}Pb generator [Sto60]. The production of the ^{204}Bi activity was carried out at the Ionenstrahllabor ISL of the Hahn-Meitner-Institut in Berlin. With the help of the ^{204}Bi/^{204m}Pb-generator TDPAC experiments on simple model compounds can be performed in our laboratory in Leipzig.

However, for our studies with biomolecules we need an ^{204m}Pb activity which is free of any competing metal contamination in order to avoid the inhibition of the targeted metal site or other side reactions.

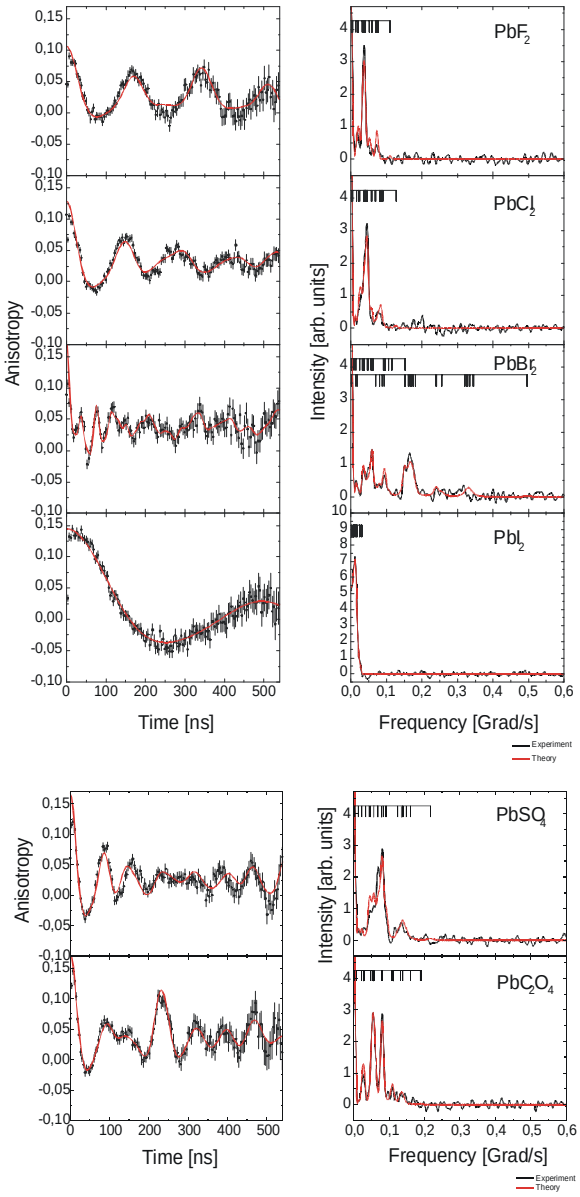

Figure 1: The TDPAC time spectra (left) and their Fourier transforms (right) of various Pb^{2+} complexes. In the Fourier spectra a frequency bar indicates the ^{204m}Pb-NQI-frequencies. With the exception of $PbBr_2$, all spectra could be fitted with one NQI. The second component in $PbBr_2$ might be due to the light sensitivity of the compound.

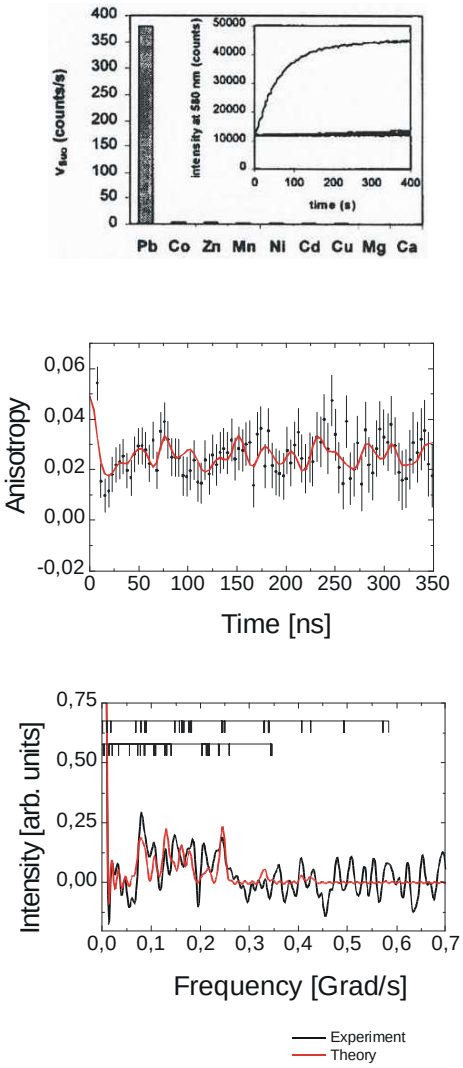

Figure 2: Top: The fluorescence response rate of the Pb^{2+} sensitive DNA. Only Pb^{2+} ions cause a drastic change of the fluorescence (from [Li00]).

Center: The TDPAC time spectrum of Pb^{2+}-DNA, the fit with two different NQIs.
Bottom: The Fourier transformed Pb^{2+}-DNA-spectra.

III. Experimental Methods

In 2002, two modern and very efficient 6-detector-TDPAC spectrometers, the so-called PAC-Cameras, were installed permanently at the Solid State Physics Laboratory of the ISOLDE on-line isotope separator at CERN. This outstation of the Leipzig TDPAC Laboratory is dedicated especially for TDPAC experiments in biochemistry and biology with rather short-

lived isomeric TDPAC isotopes, like ^{111m}Cd, ^{199m}Hg, and ^{204m}Pb with half-lifes of less or about one hour.
The isolation, purification and characterisation of the biomolecules will be done at the home laboratory at the University of Leipzig or at the laboratory of our collaboration partners. Furthermore, the metal to protein stoichiometry will be determined by µ-PIXE (Particle Induced X-Ray-Emission) at the LIPSION facility in Leipzig. The samples will be transported to ISOLDE on dry ice (≈ -80 °C).
The proposed experiments require the implantation of ^{111m}Cd,^{199m}Hg, and ^{204m}Pb for 40 to 70 minutes into ice at 100 K (in order to guarantee the vacuum in the beamline). In the last years we developed our own target holder, cooled by liquid nitrogen, at the collection point. Occasionally, we subsequently add inactive carrier (Cd(II)-, Hg(II)-, and Pb(II)-salts dissolved in adequate buffer solutions) to the molten ice to increase the metal concentrations from picomols to nanomols. After the incubation of the biomolecules with the ^{111m}Cd-, ^{199m}Hg-, and ^{204m}Pb-solution with or without carrier for 5 to 30 minutes the biomolecules are immobilized by precipitation/centrifugation, freezing, or adding sucrose. For the final preparation steps one of the hoods of the Solid State Physics Laboratory is required. There is also enough space for the cooling centrifuge and a cryobox (for storing the proteins).
Due to the rather short half-life of the isomeric TDPAC isotopes the collection point, the PAC-Cameras and the Solid State Physics Laboratory have to be as close as possible to each other, i.e. the best collection points for us would be close to the stairs in the experimental hall of ISOLDE.
There are no safety risks: all chemicals used are non-toxic due to the very low concentrations. There are no inflammable, explosive chemicals.
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IV. Proposed Experiments

The TDPAC experiments with the isomeric TDPAC probes ^{111m}Cd, ^{199m}Hg, and ^{204m}Pb will focus on the following topics:

1. <u>The "new" TDPAC-probe ^{204m}Pb:</u> Exploration of the optimal binding conditions of Pb^{2+} ions in aqueous solutions to biomolecules at different Pb^{2+} concentrations ranging form trace amount up to µ-molar concentrations. Choice of pH, buffer and a convenient Pb^{2+}-salt as a carrier with a stability constant low enough to bind to the metal sites in proteins and high enough to avoid competing side reactions.

2. <u>Blue Copper Proteins</u>, a family of globular electron transfer proteins, contain one copper ion per molecule, which is usually classified as "Type 1 Cu". TDPAC studies of several small blue copper proteins like azurin, plastocyanin or stellacyanin and other proteins containing the Type 1 Cu show that the electric field gradient tensor (EFG) components at the Cu site scale upon Cd/Hg exchange by a factor of ≈ 4 [Tro97], indicating that this metal site is rather rigid against torsions and that the geometry of the metal site is predetermined by the rigid peptide conformation of the protein and not by the stereochemistry of the metal ion

("rack induced bonding" [Gra83]). In order to corroborate this finding TDPAC experiments with a third metal ion, $^{204m}Pb^{2+}$, are required.

3. Metallothioneins (MT) are ubiquitous, cysteine-rich proteins of low molecular weight which bind d^{10} metal ions such as Zn(II), Cd(II), Cu(I), Hg(II) an Pb(II) in metal thiolateclusters [Vas]. They play an important role in the metabolism and in the modulation of the essential trace elements zinc and copper and in the binding of toxic heavy metals like Hg^{2+} [Tro01a] or Pb^{2+}. The latter suggests also the involvement in cellular detoxification mechanisms. Whereas 3D structures (NMR and X-ray diffraction) of Cd and Zn-MT are reported, the Pb^{2+} coordination in MT is unknown. The titration of high purity recombinant MT, with either $HgCl_2$ or $Hg(ClO_4)_2$, at pH 7 or 3, revealed mainly two- and fourfold Hg(II) coordinations depending on Hg(II) concentration and pH. At pH 3 twofold coordinations dominate whereas at pH 7 also higher coordination numbers occur [Tro01a, Lei03]. We will perform ^{111m}Cd- and ^{204m}Pb-TDPAC studies in order to elucidate the Cd^{2+}- and Pb^{2+} coordination in metallothioneins at different pH values and concentrations.

4. The investigation of the coordination and the dynamics of the Pb binding site in new catalytic DNA molecules which can be used as metal sensitive bio sensors in order to optimise their efficiency and selectivity as metal sensors [Li00]. Other specially designed DNA samples will be tested for their affinity to the heavy metals Cd and Hg.

These experiments will be accompanied by TDPAC experiments with model compounds and ab-initio EFG calculations in order to elucidate the coordination geometry and the binding partners. With the help of site directed mutagenesis and other biochemical methods certain metal ligands can be replaced by others and their influence on the NQI reflects the functional importance of this metal ligand.

V. Beam Time Request

We ask for a total of 28 shifts within 2 years to be used as follows:

2003:	^{111m}Cd (Sn Plasma Source):	4 shifts
	^{199m}Hg (Pb Plasma Source):	4 shifts
	^{204m}Pb (UC, LIS):	6 shifts
2004:	^{111m}Cd (Sn Plasma Source):	4 shifts
	^{199m}Hg (Pb Plasma Source):	4 shifts
	^{204m}Pb (UC, LIS):	6 shifts

The number of shifts per year of the three isotopes corresponds to one beam time per isotope and year.

We stress the fact that we do need the beam every two to three hours only. Therefore, we can share the isotope beam with other users. Furthermore, the gained experience in 2002 has shown that both separators, the GPS and the HRS, can be operated simultaneously. Hence, three experiments can be conducted contemporaneously.

In order to use the allotted beam time as efficiently as possible we have permanently installed PAC-Cameras at the Solid State Physics Laboratory at ISOLDE. If required, two other PAC-Cameras from Leipzig can be transported to ISOLDE.

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