

EXAFS investigation of quasi-covalent metal labels of proteins and polymers for ASAXS and MAD phasing

T. Nawroth¹, R. Gebhardt¹, K. Zwicker² and G. Goerigk³

Institut für Biochemie, Gutenberg-Universität, Becherweg 30, D-55099 Mainz, Germany : <http://www.MPSD.de>

¹ Institut für molekulare Biophysik, Gutenberg-Universität, Welderweg 30, D-55128 Mainz, Germany

²ZBC Zentrum Biologische. Chemie, Univ.Klinik, Theodor-Stern Kai 7, Haus 75, D-60598 Frankfurt/M, Germany

³Forschungszentrum FZ, IFF Institute for solid state physics, Postfach 1913, D-52425 Jülich, Germany

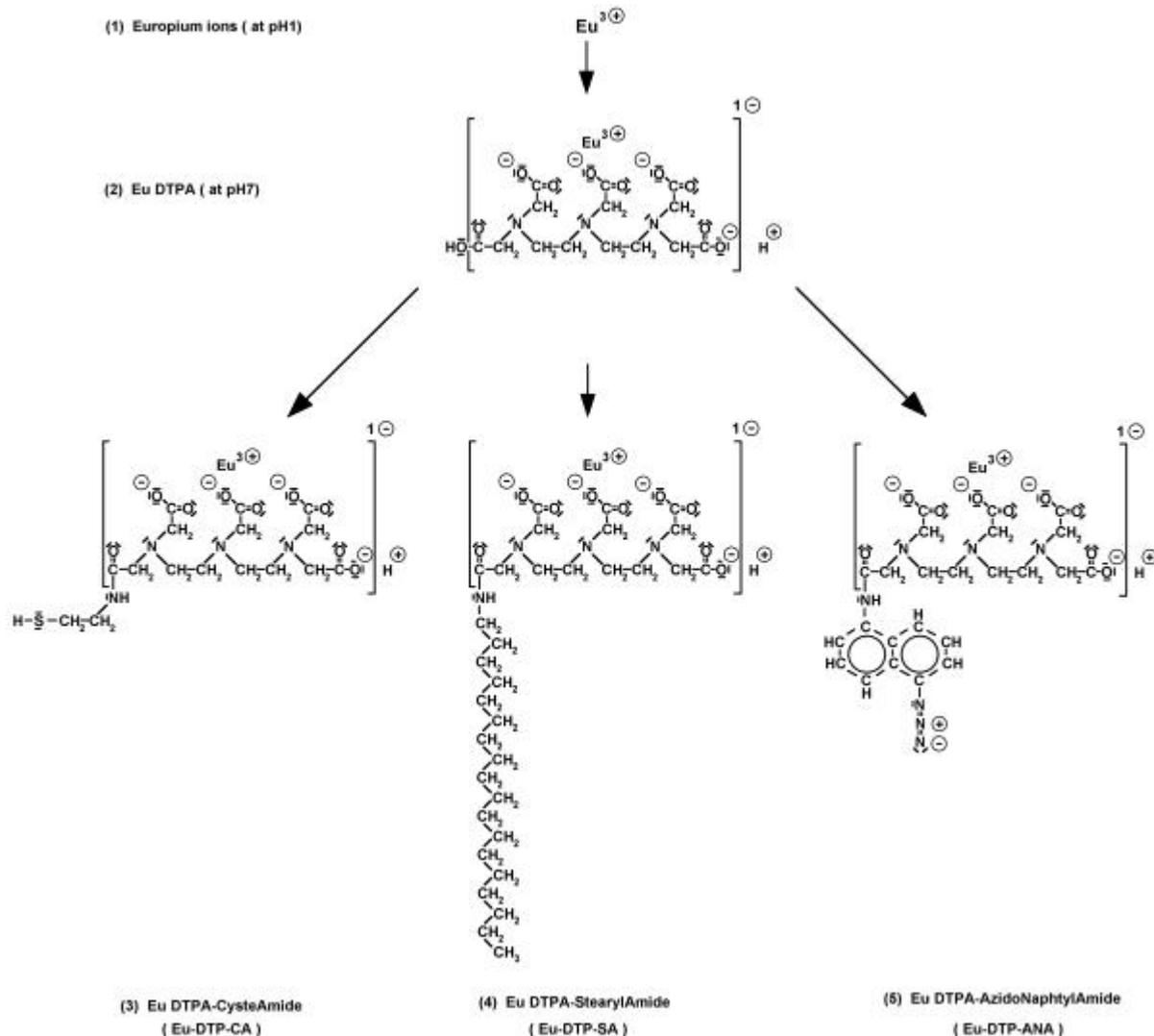


Figure 1: Anomalous scattering metals as lanthanides (Europium) can be associated to proteins and polymers after packing into highly stable chelate complexes, e.g. DTPA (Di-ethyleneTri-aminePentaAcetic acid). The pure chelate (2) can be used as counterion, while the larger derivatives (3) to (5) bind specifically to structure.

Lanthanides and other heavy metals are known as favorite structure reporters in material science, e.g in the investigation of alloys by anomalous scattering (ASAXS), anomalous diffracton (MAD phasing), absorption spectroscopy (EXAFS/ XANES), Mößbauer spectroscopy (with ⁵⁷Fe) and magnetic investigations as NMR (with paramagnetic ions like Gd, Sm). Unfortunately those ions are rare in biological matter and difficult to introduce artificially. This is due to the possible unspecific surface binding of the pure heavy metal ions to the macromolecule surface and to the “hydrolysis”, i.e. the formation of hydroxides and oxides with water:



The hydrolysis of water converts the free ions, which are stable in acidic solution (pH<1), to heterogenous mixtures of several oxy-compounds at neutral pH. This may abolish the favourable properties of macromolecular heavy metal complexes for structure investigation by formation of inhomogenous mixtures [1].

We have developed a “pH-shift helper chelator” procedure for the preparation of homogenous heavy metal DTPA complexes. As depicted in Fig.1, the Lanthanide is transferred from pH1 to pH7 during the process. Due to the extremely slow kinetics of lanthanide ligand exchange, the ion is first reacted with a weak helper chelator (citrate) at pH1 for 3 days, then shifted slowly to pH 5 during 48 h and then transferred to the more stable final chelator compound. As final step the label chelate is shifted during 6 h to neutral pH (7). As shown in Fig.1 we use several classes of metal chelates: (2) metal-DTPA is used as anomalous counterion; while the complex compounds (3)-(5) are structure specific lantern labels, which were synthesized as published earlier for dye-labels [2]: The reactive thiol compound (3) DTP-CysteAmide can be coupled to SH-groups in proteins and polymers, the hydrophobic lipid analogue (4) DTP-StearylAmide binds to membranes, while the hydrophic photolabel (5) DTP-AmidoNaphthylAzide reacts with proteins after UV-irradiation.

At the JUSIFA (B1) beamline at HASYLAB we have investigated the EXAFS spectra of EuDTPA with the “true reference method” described earlier [3] as a first step for ASAXS studies of proteins, polymers and membranes (liposomes). As a further improvement the samples and reference buffer were subjected to X-rays in a flat window flow-through cell (8 mm open diameter, 1.5 mm sample pathlength). The windows consisted of 15 μm Nalophan BF (Kalle AG, Wiesbaden). The vacuum was held by an extra window set (the 4-window cell is an improvement of the earlier helium-flushed flow through cell for ASAXS [4]). The spectrum shown in Fig.2 for a 50 mM EuDTPA solution in 0.2 M Tris-citrate buffer, pH7, was obtained despite of the low metal content (0.76%). The absorption yielded no evidence for inhomogenous ligand formation, which would interfere with the forthcoming ASAXS investigations. The region of interest of those compounds for ASAXS and MAD phasing is the left shoulder of the LIII-edge (bright white line) at 6.96 ... 6.99 keV (f' minimum).

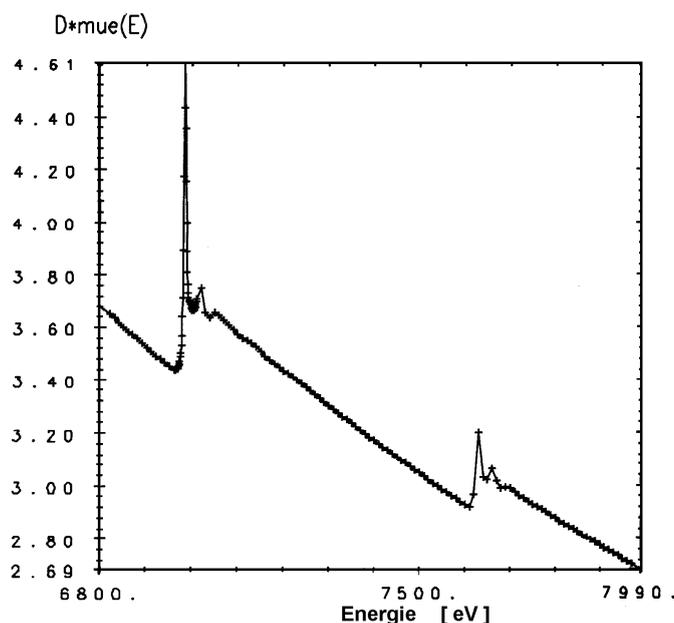


Figure 2: The EXAFS spectrum of 50 mM EuDTPA solution shows the LIII and LII absorption edges while the sample contained only 0.76% metal. The bright white line of the LIII-edge is favourable for structure labeling. The spectrum was estimated at the B1-beamline with a novel flat window flow-through cell (8mm).

References

- [1] J.-C.G.Bünzli, and G.R.Choppin, “Lanthanide probes in Life Sciences”, Elsevier, Amsterdam (1989)
- [2] T. Nawroth, Fresenius Z. Anal. Chemie 343, 173 (1992)
- [3] T. Nawroth, R. Gebhardt, H. Decker, and G. Goerigk, HASYLAB report part I, 865 (1999)
- [4] T. Nawroth, A. Neidhardt, K. Dose, O. Kühnholz, M. Hütsch, H.B. Stuhmann, HASYLAB report 326 (1987)