

# Nuclear inelastic scattering of synchrotron radiation on metmyoglobin

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## Introduction

The function of a protein is determined by its structure and dynamics. Of special interest is the dynamics at the active center of a protein. For iron-containing proteins like myoglobin, the <sup>57</sup>Fe Mössbauer spectroscopy in energy and time domain proved to be a good tool to analyze structural fluctuations and relaxations which couple to the iron center [1, 2]. In recent years, phonon-assisted Mössbauer absorption of synchrotron radiation has been used for the investigation of inorganic material [3, 4]. We applied this method to proteins (e.g., myoglobin) where only one iron belongs to a molecule of 1250 non-hydrogen atoms. This contribution deals with the investigation on dry and wet metmyoglobin. The aim is to compare the influence of a protein water shell on the vibrational spectrum at the iron site in the interior of the molecule.

## Methods and Materials

The experiments have been performed at the SRI-CAT beamline 3-ID. Behind a C(111) double monochromator and a Si(975)/Si(975) high-resolution monochromator, the incoming energy was 14.413 keV with a bandwidth of 0.85 meV. Radiation with an energy mismatch to the Mössbauer resonance level can nevertheless be resonantly absorbed by the creation or annihilation of phonons of proper energy during the absorption process. The reemission takes place with a characteristic delay time of  $\tau_c = 141$  ns for <sup>57</sup>Fe. The amount of delayed quanta at a certain energy mismatch,  $\Delta E$ , is a measure for the phonon density of states at this energy coupling to the iron site. At a synchrotron current of 80 mA within the 25×3 + 1×6 bunch mode the sample scattered about 50 delayed quanta per second in the elastic peak maximum. Scattered quanta had been detected by an avalanche photo diode with a time resolution better than 1 ns. The high-resolution monochromator was tuned in the +/-70 meV energy region in steps of 0.28 meV. The sample was held in a closed-cycle He cryostat.

For the investigations sperm whale metmyoglobin was used, enriched in <sup>57</sup>Fe according to Teale [5]. The myoglobin solution was purified with a cation exchange chromatography column (SP-sepharose) on a Pharmacia FPLC equipment. From the purified myoglobin solution, the buffer was exchanged with twice-distilled water, the solution lyophilized with silica gel, and vacuum dried perfectly. From this dry sample, a part was rehydrated by storing it in a atmosphere of high humidity until the water content

reached the same amount as found in myoglobin crystals (0.38 g H<sub>2</sub>O/g protein). Dry and wet samples were kept in vacuum-tight sample holders.

## Results

Spectra of dry metmyoglobin were measured at T = 304 K and 145 K, spectra of wet metmyoglobin at T = 300 K, 235 K, 170 K and 45 K. As an example, Fig.1 shows the phonon density of states (DOS) for the room-temperature data extracted by the Fourier-log method [6].

## Discussion

Figure 1 shows that the density of phonon states coupling to the iron in wet and dry metmyoglobin is nearly identical in the energy regime less than 20 meV. However, at higher energies clear differences become visible. The mode at 33 meV is strong in wet metmyoglobin and seems to be nearly suppressed in dry metmyoglobin where an additional mode is found at 42 meV. Following the interpretation that the mode at 33 meV is structural sensitive to the heme moiety [7], this indicates some structural changes at the iron site in the interior of myoglobin caused by removing the water shell of the protein molecule. Details of the analysis will be given in a forthcoming paper [8].

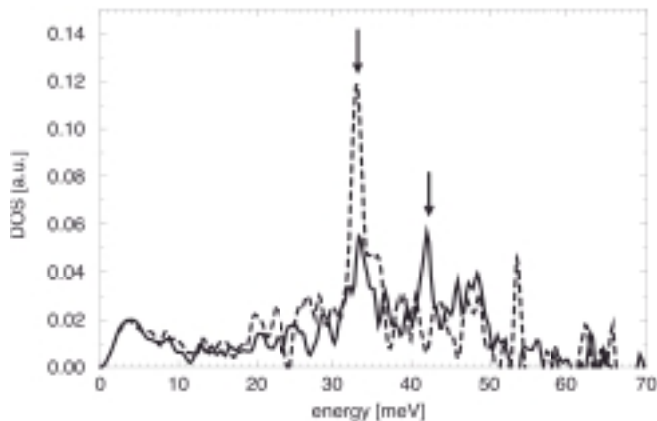


Figure 1: Density of phonon states coupling to the iron in metmyoglobin. Solid line: dry sample, dashed line: hydrated sample with 0.38 gH<sub>2</sub>O/g protein. The arrows mark phonon vibrational modes mentioned in the text.

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