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Report:

Temperature dependent inelastic X-ray scattering on myoglobin studied by nuclear resonance energy analysis

A new method /l/ of inelastic X-ray scattering with an energy analysis by the Mossbauer effect was used to measure the dynamics of myoglobin /2/. The method is based on the following principle: X-rays from a synchrotron are incident on the sample under investigation and scattered elastically and inelastically. The incoming radiation has an energy of about 14.4 keV and a band width of 4.4 meV. The energy can be changed in steps of 0.16 meV in the range of +70 meV. In an inelastic scattering process a X-ray quantum gains or loses energy by the interaction with modes of motions in the sample. If this energy change is just the right one to reach the Mossbauer resonance energy of ^{57}Fe, the radiation scattered by the sample can be furthermore resonantly forward scattered /3/ in an analyzer containing ^{57}Fe nuclei. This nuclear scattering leads to time delayed radiation, the intensity of which is proportional to the density of motional modes with proper energy.

An amorphous sample of horse heart myoglobin hydrated to 0.40 g water / g protein was investigated. It was contained in a sample holder with vacuum tight mylar windows and mounted in a closed cycle refrigerator. The energy dependence of the inelastic X-ray scattering by this sample was measured at temperatures T = 62 K, 124 K, 189 K and 300 K (see Fig. 1). The scattering geometry /1,2/ allowed for the detection of radiation with momentum transfers k in the sample between 2.6 $Å^{-1}$ and 14.4 $Å^{-1}$ with a maximum at 3.9 $Å^{-1}$.

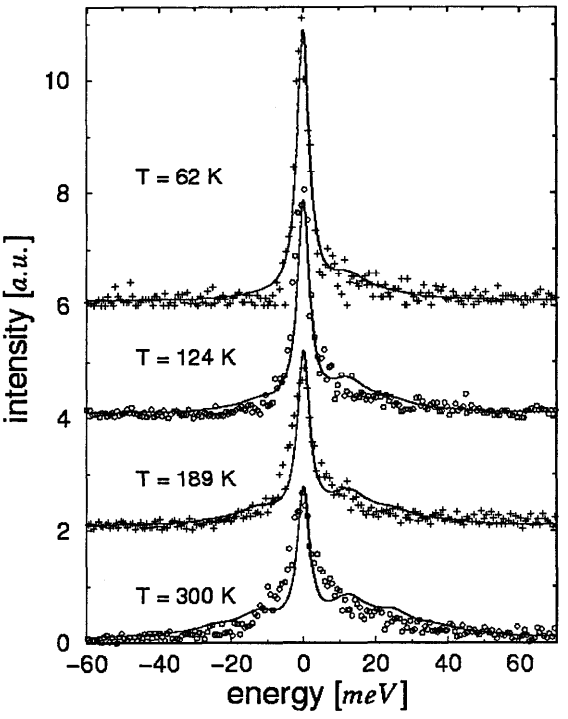

Fig. 1 Energy spectra of inelastic X-ray scattering by hydrated myoglobin at four temperatures. The solid lines are calculated spectra using an Einstein frequency obtained from a normal mode analysis of myoglobin.

The energy spectra were approximated within an Einstein model using results of a normal mode analysis of myoglobin /4/. This simple model describes the spectra asthonishingly well up to T=189 K with an atomic mean square fluctuation given by $< \overline{R^2} > = 3.53 \cdot 10^{-4}$ $Å^2/K \cdot$ T. It shows that at these temperatures the dynamics can be explained by harmonic vibrations. The spectrum at T = 300 K could only be approximated by taking into account additional modes of motions leading to $< \overline{R^2} > = 4.33 \cdot 10^{-4}$ $Å^2/K \cdot$ T. These findings show the importance of protein specific motions above 200 K in myoglobin. They are in accordance with results obtained by Mossbauer spectroscopy (sensitive to ^{57}Fe) /5/ and by incoherent neutron scattering (sensitive predominantly to H-atoms) /6/. The new method, by contrast, gives mainly information on the C,N and O-atoms. A more detailed analysis of the experimental results is in progress.

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