Far-Infrared Emission by Boson Peak Vibrations in a Globular Protein

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The Raman- and neutron-scattering spectra of proteins and supercooled liquids display a common feature at low frequency, the boson peak. We elucidate its microscopic nature in relation to biological activity and the glass transition. Our experiments show that optical pumping of a heme protein leads to nonthermal emission in the far-infrared related to boson peak vibrations. The vibronic relaxation of the heme group channels energy into far-infrared modes which are damped with temperature and hydration. The results are consistent with a viscoelastic model of boson peak excitations. [S0031-9007(99)08836-5]

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One of the basic questions of biomolecular dynamics concerns the statistical time correlation of structural and functional variables [1,2]. Most globular proteins are composed of one or several functional sites which are shielded from the surrounding solvent by a highly folded and closely packed polypeptide chain. However the biological activity of these molecules also involves the exchange of substrate and product molecules between the protein interior and the solvent, which requires a limited degree of structural flexibility. Several normal mode studies suggest that the conformational path between open and closed forms of these proteins proceeds via a few delocalized low-frequency modes in the range below 20 cm^{-1} [3,4]. The corresponding vibrational frequencies fall in the range of fast dissipative processes which raises doubts whether such oscillations can persist for several periods.

The Raman- and neutron-scattering spectra of hydrated proteins generally exhibit a broad central line with a weak shoulder between 15 and 30 cm^{-1} [5,6]. With decreasing temperature [7,8] or degree of hydration [9] the central line narrows, while the shoulder develops into a broad peak. These results could be reproduced qualitatively by molecular dynamics simulations [10,11] of a single myoglobin molecule, suggesting an assignment to intramolecular modes. The peak proved to be unspecific to protein structure and folding but can be easily perturbed by changes in hydration or solvent composition.

In this Letter we draw attention to the remarkable similarity of the Raman- and neutron-scattering spectra of proteins with those of supercooled liquids and glasses [12]: The conspicuous broadband in unstructured systems has been called the "boson peak" [13] and is a characteristic feature of low molecular weight glasses which is not observed in the spectra of the corresponding single crystals. Early explanations were based on the concept of disorder-induced scattering [14]. A recent model describes a system of coupled classical oscillators with spatially fluctuating nearest neighbor force constants [15]. Molecular simulations [16] and neutron-scattering experiments [9] of proteins indicate a damped librational character of side-chain oscillations at low frequencies. Thus the broadband can be understood as a manifestation of the elastic limit in the viscoelastic behavior of liquids. Generalized hydrodynamics accounts for the finite relaxation time of dissipative processes [17]: The friction coefficient becomes frequency dependent, decreasing at high frequencies. In molecular hydrodynamics the dynamic structure factor $S(q,\omega)$ for each mode q with frequency $\Omega(q)$ is the imaginary part of $S(q)\Phi(q,\omega)$, where [8,18]

$$\Phi(q,\omega) = -\frac{\omega + \Omega^2(q)M(q,\omega)}{\omega^2 - \Omega^2(q) + \omega\Omega^2(q)M(q,\omega)}. \quad (1)$$

$S(q)$ denotes the static structure factor. $M(q,\omega)$ represents a generalized friction which can be decomposed into a Newtonian friction $\nu(q) = \Omega^2(q)\gamma(q)$ and a relaxing part $m(q,\omega)$,

$$M(q,\omega) = i\gamma(q) + m(q,\omega). \quad (2)$$

In neutron-scattering experiments one directly probes the dynamical structure factor which contains coherent and incoherent contributions. The incoherent cross section of the protein hydrogens dominates the scattering, which leads to an approximately q independent line shape of the spectra at low frequency [19].

Figure 1 shows the neutron-scattering function $S(q,\omega)$ at $q = 1.7$ Å^{-1} of lyophilized myoglobin at 150 and 300 K and a spectrum of D_2O-hydrated myoglobin at 180 K for comparison. The experiments were performed with the time-of-flight spectrometer IN 6 at the Institute Laue-Langevin in Grenoble using a neutron wavelength of 0.51 nm. To compensate for a harmonic temperature dependence the low temperature spectra were scaled to 300 K using the Bose occupation factor, $n(\omega,T) = 1/[\exp(\hbar\omega/k_BT) - 1]$. The spectra superimpose above 3 meV as expected of harmonic behavior. The data at 150 K display a well-defined minimum between the resolution-limited elastic peak at $\omega = 0$ and the maximum of the boson peak at 2.5 meV. Note that the spectrum of the hydrated system has less intensity at low frequency in relation to the dehydrated case. We assign this difference to strong protein-water hydrogen bonds which depress the librational motions of the polar side

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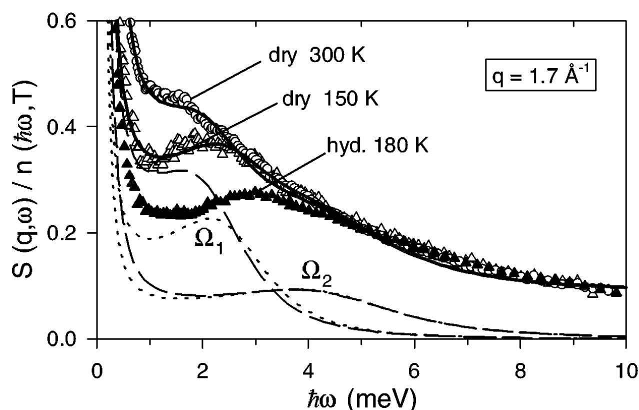

FIG. 1. Quasielastic neutron-scattering spectra of dry myoglobin at 150 and 300 K and a two mode fit to the viscoelastic model of Eq. (1). Parameters: $F_1(150\ \mathrm{K}) = 1.2$, $F_1(300\ \mathrm{K}) = 0.7$, $F_2 = 2$, $\tau_{1,2}^{-1}(150\ \mathrm{K}) = 0.09$ meV, $\tau_{1,2}^{-1}(300\ \mathrm{K}) = 0.18$ meV, $\gamma_1 = 0.7\ \mathrm{meV}^{-1}$, $\gamma_2 = 0.55\ \mathrm{meV}^{-1}$, $\Omega_1 = 1.9$ meV, and $\Omega_2 = 2.8$ meV; a constant of 0.09 was added and the components were weighted by 0.7 (1) and 1.5 (2). Data of hydrated myoglobin at 180 K are shown for comparison.

chains. Increasing the temperature to 300 K leads to quasielastic broadening of the central line filling the minimum between the central line and the boson peak. The quasielastic intensity evolves at the expense of the elastic central line and not by an apparent softening of the boson peak vibrations. To probe whether a viscoelastic model can account qualitatively for the temperature evolution of the spectra, we adjust the theoretical curve [Eq. (1)] at 150 K using a superposition of two modes Ω_1, Ω_2.

For simplicity we also assume a q-independent Lorentzian line shape for the spectrum of the friction kernel, $m(\omega) = -F/(\omega + i/\tau)$ which is equivalent to Maxwell's viscoelastic model of liquids. F denotes the area of the Lorentzian and τ is the correlation time of the relaxing friction. τ is adjusted to 7 ps which corresponds to the width of the resolution-limited elastic line. To model the spectrum at 300 K we adjust τ to 3.5 ps. F was reduced from 1.2 to 0.7 consistent with some mode softening. The other parameters were kept constant. The resulting spectrum reproduces the 300 K data rather well. Note that the low-frequency vibration Ω_1 is much more affected by the relaxing friction than the second mode at Ω_2. The model mimics a common observation with boson peak spectra which display nearly harmonic temperature dependent high frequency wings.

Boson-type modes were seen with some heme proteins directly in the time domain: In genetically modified bacterial reaction centers damped oscillations in the nuclear coordinates of an excited state were recorded at low temperatures. The Fourier analysis revealed the presence of a low-frequency component at 15 cm^{-1} [20], which was attributed tentatively to structural modes. The oscillatory behavior disappeared above the glass temperature of the solvent. However with deoxy-myoglobin underdamped oscillations at 75 cm^{-1} using optical femtosecond pulses were reported to persist at room temperature for several periods. The feature was assigned to a doming mode of the planar heme group [21,22]. Most interesting, Austin *et al.* [23] could perturb the reaction rate of CO with the heme group of myoglobin using the far-infrared radiation of a free electron laser which was tunable from 45 to 55 cm^{-1}. The nonthermal rate enhancement disappears above the glass temperature of the solvent. In principle, the kinetic perturbation could result from specific excitation of heme doming modes, but this assignment would leave the requirement of low temperatures unexplained. Alternatively one could consider a tight coupling between vibronic relaxation of the heme and structural modes in the boson peak region which become overdamped at high temperatures.

We address the question of damping directly by exploiting the suggested coupling of the heme to structural modes of myoglobin. We excite the iron porphyrin by visible light in the Q-band region at 532 nm. The electronic excited state decays on a subpicosecond time scale by vibronic relaxation; the fluorescence of iron porphyrins is strongly quenched [21]. A fraction of the energy will excite low-frequency protein modes which, depending on the degree of damping, may exhibit radiative decay in the far infrared (FIR). The FIR channel competes with fast dissipative processes. The emitted FIR intensity can thus serve as a sensitive monitor of viscous damping in the boson peak region.

In the relevant wavelength range, 200–500 μm broadband, fast pyroelectric transducers can be employed as detectors. The main drawback is their poor sensitivity (0.25 μA/W, Molectron P1-15). To discriminate the signal from a slowly varying FIR background, we excite the protein using the spatially expanded 10 ns pulse of a frequency-doubled Nd:YAG laser at 532 nm. To select the FIR signal, we use a bank of filters: (1) a 3 mm thick fused silica substrate which carries the sample. Fused silica is transparent in the visible and near infrared up to 4.5 μm and again in the FIR, 150 to >1000 μm [24,25]. (2) A 1 mm thick germanium plate which is transparent in the near (>1.9 μm) and far infrared serves to eliminate the green light of the laser pulse. (3) To block the near infrared we employ 0.1 mm of carbon blackened polyethylen foil, transparent from 25 to >1000 μm [26]. The resulting low pass filter has a transmission of 7% above 150 μm and less than 10^{-5} at shorter wavelengths measured with a Bruker IFS 113v FT-IR spectrometer. After amplification, the signal is monitored and averaged (100 shots) with a digital oscilloscope (LeCroy 9310).

Films of metmyoglobin (horse muscle) were prepared by drying 200 μl of a concentrated protein solution (1 mM, phosphate buffered to pH 7.6) on the substrate plates in a desiccator containing silica gel. Hydrated samples (0.35 g/g) were by obtained storing the films

four days at 90% humidity over a saturated sodium chloride solution. At 532 nm, the optical absorption of the samples was 75%. Thus 32 mJ of the 43 mJ laser energy were absorbed by the sample. The resulting heating effect of the sample does not exceed 3 °C.

Figure 2 shows the sensor response for a dry myoglobin film. The emission is prompt and occurs only during the 10 ns of the exciting laser pulse. This result is to be expected considering that the lifetimes of the protein structural modes are in the range of a few picoseconds. The time signature demonstrates that we are observing a nonthermal effect. A prompt temperature step by 3 °C as mentioned above would produce a step in the thermal emission. Furthermore, our signal is by serveral orders in magnitude larger than the one expected as an upper limit of such a temperature increase according to the Stefan-Boltzmann law. We also carefully eliminated the possibility of leakage of green light hitting the detector and the contamination by electronic noise. We subtract a "dark" spectrum from the data to remove electric interference. Neither the empty cell with all the windows nor carbon black as a sample produced any signal. Further the observed peak amplitude of the detected signal changes in a reproducible manner when the temperature is cycled. To measure the total emitted FIR energy we integrated the sensor response with a short time constant (150 ns) to overcome the limited sampling rate (100×10^6 samples/sec) of our oscilloscope. The inset shows how the peak amplitude of the integrated signal, which is proportional to the total emitted FIR energy, varies with temperature and hydration. The detector-amplifier system was calibrated with attenuated light pulses of known energy (Ophir joulemeter) in the visible range. The detector sensitivity is wavelength independent.

Figure 3 shows the energy ratio of the FIR emission relative to the absorbed energy of the incident laser pulse. For the dry sample this fraction remains constant at temperatures below 200 K. The FIR intensity decreases above 200 K reaching a level of 50% at 320 K. The sample was then hydrated to 0.35 g/g and reexamined afterwards in the same setup. At 300 K we observe approximately the same signal as in the dehydrated case, which increases slightly with decreasing temperature. At low temperatures however, we find that the FIR signal of the hydrated protein levels off at a significantly lower value. Furthermore, an exposure of the hydrated sample to temperatures above 320 K leads to a decrease in the signal. Optical inspection of reference samples shows that the protein denatures in this temperature range. These results can be understood in the context of neutron-scattering studies of dry and hydrated myoglobin: The boson peak is depressed in the hydrated system by strong protein-water hydrogen bonds at low temperatures (Fig. 1). This implies a reduced vibrational amplitude which is consistent with a lower FIR intensity observed for hydrated myoglobin below 200 K. Above 200 K the neutron-scattering spectra exhibit increasing quasielastic broadening as displayed in Fig. 1 for a particular temperature [8,9]. The assignment of this effect to a particular molecular process is not straightforward. However, the FIR emission decreases with increasing temperature. This result together with the model calculations presented in Fig. 1 support a viscoelastic line-broadening mechanism. We thus assume that the radiative decay competes with dissipative processes. To estimate the radiative fraction f_r we relate the vibrational frequency $\Omega_r = 0.5/\mathrm{ps}$ to the rate of dissipative decay k_{diss}. For the latter we assume an Arrhenius temperature dependence, $k_{\mathrm{diss}} = k_0 \exp[-H/(RT)]$,

$$f_r = \mathcal{F} \frac{\Omega_r}{\Omega_r + k_{\mathrm{diss}}}. \tag{3}$$

$\mathcal{F}$ denotes the vibronic coupling coefficient assumed to be independent of temperature. The fit shown in Fig. 3 yields an activation enthalpy H of 11 (± 2) kJ/mol

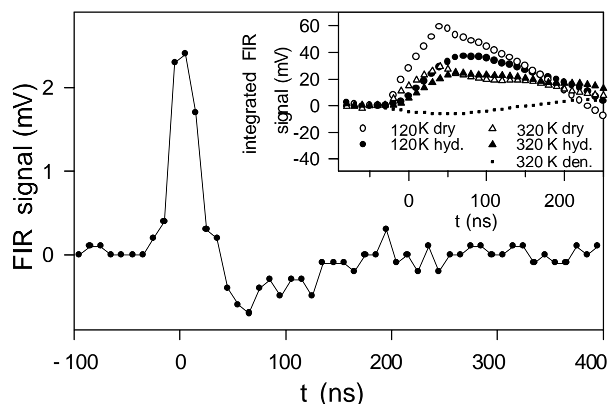

FIG. 2. Detector signal for dry myoglobin at 130 K. The inset shows the integrated signal, whose peak amplitude is proportional to the emitted FIR energy, for dry and hydrated myoglobin at 120 and 320 K and for the denaturated sample.

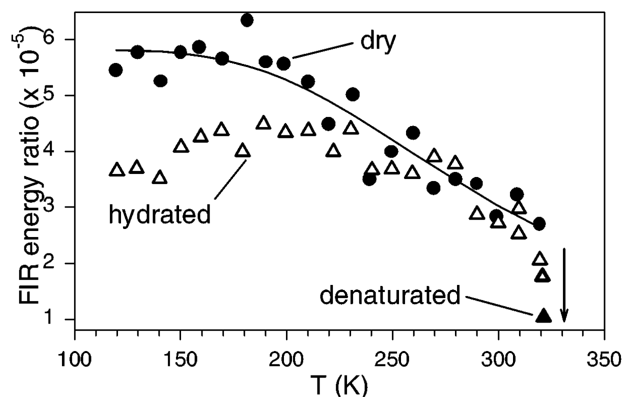

FIG. 3. Ratio of the total emitted FIR energy to absorbed laser energy for dry and hydrated myoglobin films. The arrow shows how the signal of the hydrated sample decreases within 1 h at 320 K. The ratio was calculated from the calibrated detector response taking filter transmission and beam geometry into account.

and $\log[k_0/(1\ s^{-1})] = 13.6\ (\pm 0.5)$ for the dissipative process. These numbers are consistent with the braking of a single hydrogen bond and similar to those derived in the analysis of the neutron-scattering spectra of hydrated myoglobin [7]. The decrease in FIR intensity observed with dry and hydrated myoglobin in the temperature range above 220 K suggests a similar degree of viscoelastic damping. One might expect that the addition of water enhances the damping efficiency. The unfolding experiment supports this view: Denaturation of the hydrated protein exposes the heme and buried side chains to water which leads to the observed loss in FIR intensity at 320 K. The nearly identical FIR intensities may indicate that intramolecular friction and water, to a lesser extent, control the damping of excitations in the boson peak region in the native protein.

To summarize, we provide evidence that the vibronic relaxation of an optically excited state of a heme protein leads to radiative decay in the FIR below 70 cm^{-1}. Since the lowest known intramolecular vibrational frequency of the heme is the doming mode at 75 cm^{-1} [21], our results point to a tight coupling of low-frequency structural modes and librational modes of the heme as a whole. The coupling mechanism may explain in part why FIR irradiation allows one to modify the ligand binding kinetics at low temperatures [23]. Our analysis supports the relevance of viscoelastic damping of modes in the boson peak region which may have implications on similar spectral features of supercooled liquids [27]. The experiment also shows that a pulsed FIR source can be built with optical lasers using the coupling of a chromophore to boson peak modes. The transformation efficiency in our setup was in the range of 10^{-5}.

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