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Measuring the elastic properties of protein crystals by brillouin scattering

C.L. Caylor[a], S. Speziale[b], S. Kriminski[a], T. Duffy[b], C-S Zha[c], R.E. Thorne[a,*]

[a] *Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, NY 14853, USA*
[b] *Department of Geosciences, Princeton University, Princeton, NJ 08544 USA*
[c] *Cornell High-Energy Synchrotron Source, Ithaca, NY 14853 USA*

Abstract

We report preliminary measurements of the elastic properties of tetragonal lysozyme crystals using Brillouin scattering. This microscopic, non-contact technique is ideally suited for study of fragile, optically transparent macromolecular crystals. Brillouin scattering should allow much more complete characterization of crystal elasticity, and provide a novel probe of intermolecular interactions, conformation changes, and defect formation.

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1. Introduction

Elastic properties are among the most important and least studied physical properties of macromolecular crystals. They determine the mechanical deformations produced in response to external and internal stresses, and control the energetics of lattice defect formation in response to molecular heterogeneity, nonuniform impurity incorporation, and post-growth treatments including ligand binding and flash freezing. The elastic properties themselves are determined by the compressibility of the macromolecule and of the intermolecular contacts, and can thus provide information about molecular conformation and crystal packing that is complementary to that obtained by X-ray diffraction.

The potential of elasticity measurements as a probe of molecular and lattice properties is suggested by the experiments of Morozov, and coworkers [1–8]. They measured the Young's modulus of glutaraldehyde cross-linked crystals of the proteins horse haemoglobin, sperm whale myoglobin, bovine serum albumin, and hen egg white lysozyme by the vibrating reed technique, in which a thin, usually microtomed section of a crystal is clamped at one end and the other end capacitively driven to vibrate. By measuring the resonant vibration frequency and knowing the exact shape and size of the sample, Young's modulus in one direction can be estimated. By measuring the resonance width, the damping and

*Corresponding author. Tel.: +1-607-255-6487; fax: +1-607-255-6428.

E-mail address: ret6@cornell.edu (R.E. Thorne).

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internal friction can be determined. Morozov et al. observed variations in Young's modulus with temperature, pH, salt concentration, isotopic composition of solution water, and humidity in both crystals and amorphous films. Edwards et al. [9] used the ultrasonic technique to measure sound velocities in ribonuclease-A and haemoglobin crystals, and used these to estimate their longitudinal elastic modulus. More recently, Holmes et al. [10] used the three-point bending technique on monoclinic lysozyme crystals to measure Young's modulus on long monoclinic lysozyme crystals and to study crack formation and propagation.

The vibrating reed and three-point bending techniques provide only limited information about elastic properties, and require crystals with rod-like geometries. More seriously, they require physical contact between these extremely fragile crystals and rigid supports; this leads to plastic distortions and/or necessitates cross-linking, both of which complicate experimental interpretation. The ultrasonic technique can in principle provide more detailed information, but requires extremely large crystals (the crystals used by Edwards et al. had volumes $>10\,\mathrm{mm}^3$) and significant sample manipulation. We describe here preliminary measurements using Brillouin scattering, an alternative and potentially much more powerful probe of macromolecular crystal elasticity.

2. Crystal elasticity and Brillouin scattering

For a general anisotropic solid, Hooke's law relating stress σ (force per unit area) and strain ε (deformation) is generalized to

$$\sigma_i = \sum_{i=1}^{6} c_{ij}\varepsilon_j \qquad (1)$$

where indices 1–3 correspond to components of normal stress and extensional strain and indices 4–6 correspond to shear components of stress and strain. The number of independent elastic constants c_{ij} depends on the crystal symmetry.

Aside from their macroscopic significance, the elastic constants also determine the dispersion relations for long-wavelength acoustic phonons (i.e., sound waves) propagating within the crystal. The one longitudinal and two shear (transverse) acoustic modes propagate at speeds $v = \omega/q$ where, for a known direction of phonon propagation, the phonon velocities can be related to the elastic constants c_{ij} by the Christoffel equation [11].

Brillouin scattering is the inelastic scattering of light by acoustic phonons [12–17]. Conservation of energy and momentum requires that the frequency of the scattered light be shifted from that of the incident light by

$$\triangle\omega = \pm 2vk\,\sin(\theta/2), \qquad (2)$$

where v is the phonon velocity in the measured direction, k is the wave vector of the incident light, and θ is the angle between the incident and scattered wave vectors. This frequency shift is typically $\backsim 10^{10}\,\mathrm{Hz}$ (or $\backsim 1\,\mathrm{cm}^{-1}$), roughly 0.001% of the frequency of the incident light. With high-performance interferometers, this frequency shift can be measured with an uncertainty of $<1\%$.

Using the Brillouin scattering-determined phonon velocities together with the Christoffel equation to solve for the elastic constants is in general difficult, but is simplified if the scattering wave vector is chosen along an axis of symmetry. For tetragonal hen egg white lysozyme crystals (space group $P4_32_12$), the elastic tensor has six unique non-zero elements. These crystals grow with flat facets perpendicular to the $\langle 1\,0\,1\rangle$ and $\langle 1\,1\,0\rangle$ directions. For 180° backscattering normal to a $\{1\,1\,0\}$ facet, the scattering phonons propagate in the $\langle 1\,1\,0\rangle$ direction. In this case, $\rho v^2 = \frac{1}{2}(c_{11} + c_{12} + 2c_{66})$ for the longitudinal mode, while $\rho v^2 = \frac{1}{2}(c_{11} + 2c_{66})$ $\rho v^2 = c_{44}$ and $\rho \mathrm{v}^2 = \frac{1}{2}(c_{11} - c_{12})$ for the two transverse modes, where ρ is the crystal's mass density. By measuring frequency shifts and sound velocities using suitable optical polarizations in carefully chosen crystal directions, the full elastic constant tensor can be determined.

3. Experimental methods and results

Crystals of tetragonal hen egg white lysozyme were grown in hanging drops from 0.1 M acetate buffer containing 60–80 mg/ml lysozyme and 0.48 M NaCl at pH = 4.5 and $T = 21^{\circ}C$. Selected

crystals with typical dimensions of 200–500 μm were mounted in square cross-section glass capillaries together with a plug of mother liquor. A neodymium vanadate laser ($\lambda = 532$ nm) operated at 1.5 W and filtered to 15 mW was focused to illuminate a small spot within the crystal. Scattered light was collected using a lens, analyzed using a six-pass Sandercock tandem Fabry-Perot interferometer, and detected using a solid state photon detector with a 70% quantum efficiency. Measurements of diffraction patterns, mosaicity, and X-ray topographs showed no evidence of crystal damage following illumination at this intensity for several hours.

To date, our measurements have all been conducted in two standard geometries: the 180° backscattering and 90°-R scattering geometries. The latter corresponds to incident and scattered rays separated by 90° and passing through the same facet, such that the scattering vector is normal to the facet. Consequently, both geometries probe phonons propagating in the same direction.

Fig. 1 shows a typical tetragonal lysozyme crystal spectrum obtained in the 90°-R scattering geometry for a scattering vector along a $\langle 1\,1\,0\rangle$ crystal direction. In both geometries, only the longitudinal acoustic phonon mode was observed.

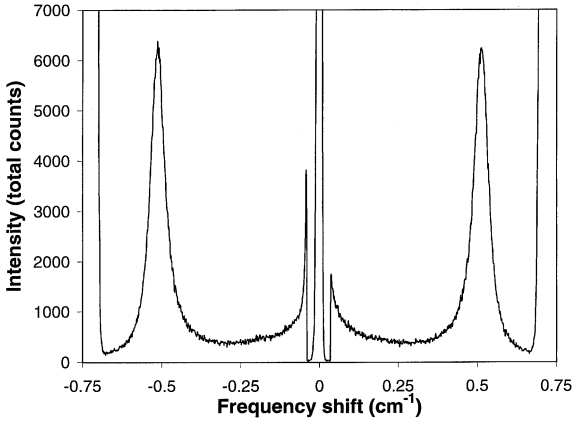

Fig. 1. Brillouin spectrum for a tetragonal lysozyme crystal acquired in the 90°-R scattering geometry with scattering wavevector along the $\langle 1\,1\,0\rangle$ direction. The peak at zero frequency is due to Rayleigh scattering.

Comparison of the frequency shifts in the two geometries yields a refractive index $n = 1.62 \pm 0.11$, in reasonable agreement with the value 1.56 ± 0.01 obtained from optical reflection measurements and with the value reported by Cervelle et al. of 1.538–1.575 [18].

From the Brillouin frequency shift of 0.42 to 0.51 cm^{-1} measured in the 180° backscattering and 90°-R scattering geometries, we obtain values for the composite elastic constant $c_{11} + 2c_{66} + c_{12}$ ranging between 6.2 and 12.6 GPa. Morozov and coworkers [1–8] reported a Young's modulus in the [0 1 1] direction of crosslinked triclinic lysozyme crystals of 1.0 ± 0.11 GPa. For crosslinked crystals of the other proteins studied, they obtained Young's moduli between 0.3 and 1.5 GPa. Holmes et al. [10] reported a Young's modulus for native monoclinic lysozyme crystals of 0.5 GPa. Values of the adiabatic bulk modulus for lysozyme, obtained by high-pressure X-ray diffraction or sound velocity measurements in lysozyme solutions, have ranged from 6.3 to 17.5 GPa [19–22]. Edwards et al. [9] obtained a longitudinal elastic modulus for ribonuclease crystals (in an unspecified direction) of 3.9 GPa. Thus, the present results obtained using Brillouin scattering are in order-of-magnitude agreement with values of elastic constants determined by other techniques. They are closer to values obtained by X-ray diffraction and ultrasonic measurements than those obtained by the vibrating reed and three-point bending techniques. However, the Young's modulus determined by these latter two techniques is related in a complex, nonlinear way to the components c_{ij} of the elastic modulus tensor [23], so that quantitative comparison will require measurement of these components.

The observed width of the Brillouin peaks is due to the convolution of the intrinsic peak width with the instrumental resolution. To first approximation, the instrumental resolution function is given by the Rayleigh peak at zero frequency. Subtracting the FWHM of this peak from those of the Brillouin peaks yields a lower bound on the $\langle 1\,1\,0\rangle$ phonon lifetime of $2.1 \pm 0.1 \times 10^{-10}$ s. This value is consistent with phonon lifetimes obtained for other organic solids at room temperature,

although other factors including static disorder can contribute to the observed peak width.

Conclusion

These preliminary results demonstrate the feasibility of obtaining Brillouin spectra of phonons from macromolecular crystals of ordinary size and shape, and using these spectra to obtain information about elastic constants. With proper choice of crystals and scattering geometry, it should be possible to measure the full elastic constant tensor and to observe both shear and longitudinal modes and their associated dampings. Because of Brillouin scattering's many advantages relative to other techniques, we believe that it has significant potential as a probe of macromolecular crystal elasticity and structure.

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