

Time-Resolved Optical Kerr-Effect Spectroscopy of Low-Frequency Dynamics in Di-L-alanine, Poly-L-alanine, and Lysozyme in Solution

Gerard Giraud and Klaas Wynne*

Department of Physics, University of Strathclyde, Glasgow G4 0NG, Scotland

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Protein functionality depends on the collective motion of its secondary and tertiary structure. Low-frequency delocalized vibrational modes in proteins could form part of the reaction coordinates of the functional motions of active proteins. While the static structure of proteins can be measured by X-ray crystallography, NMR, circular dichroism, or FTIR experiments, the picosecond delocalized motions of proteins are harder to observe especially in solution. In principle, the low-frequency dielectric response of proteins can be accessed directly via far-infrared or terahertz (THz) absorption spectroscopy. However, the study of proteins by THz spectroscopy is currently limited by two factors. First of all, the semiconductors used to generate THz radiation are opaque at frequencies between 100 and 200 cm^{-1} because of phonon-band absorption.¹ Second, water absorption at THz frequencies prevents any meaningful study of proteins in aqueous solution. A possible alternative to THz spectroscopy is optical heterodyne-detected Raman-induced Kerr-effect spectroscopy (OHD-RIKES). This technique has proven to be far superior to Raman spectroscopy at low frequencies ($<300 \text{ cm}^{-1}$), and its high signal-to-noise ratio allows a detailed analysis of the spectra. Here, we present for the first time OHD-RIKES spectra of peptides and protein in solution.

To study the conformational dependence of the low-frequency spectra of proteins, three systems of increasing structural complexity were investigated: the dimer di-L-alanine (ALA(2)), the α -helical peptide poly-L-alanine (PLA), and the predominantly α -helical protein lysozyme. PLA has very low solubility in aqueous and organic solvents but does dissolve well in organic acids such as dichloroacetic acid (DCA) and trifluoroacetic acid (TFA).² The bulky CHCl_2 group of the DCA molecules prevents the breaking and unfolding of the α -helical peptide.^{3,4} Therefore, the configuration of PLA in DCA is α -helical with 3.6 residues per turn as confirmed by Raman optical-activity measurements.⁵ PLA (mol. weight 1–5 kDa \approx 30 residues) was obtained from ICN Biomedicals, and ALA(2) and lysozyme were obtained from Sigma-Aldrich and were used without further purification. Typically, 0.2 g of dry protein was dissolved in 0.5 mL of DCA. TFA is not such a good solvent, and only 0.1 g of dry protein could be dissolved for the same volume of solvent. Filtering with syringe filters (0.22 μm pore diameter, Millipore) was essential to prevent light scattering in the sample.

OHD-RIKES experiments have been reported previously.⁶ This polarization-spectroscopy technique provides a signal linear in the third-order nonlinear optical response of the sample. Information is obtained regarding ultrafast solvent dynamics by measuring the time response of a transient birefringence that is induced in the sample by a polarized femtosecond optical pulse. Our setup uses very short (20 fs) stable laser pulses, and the signal is obtained

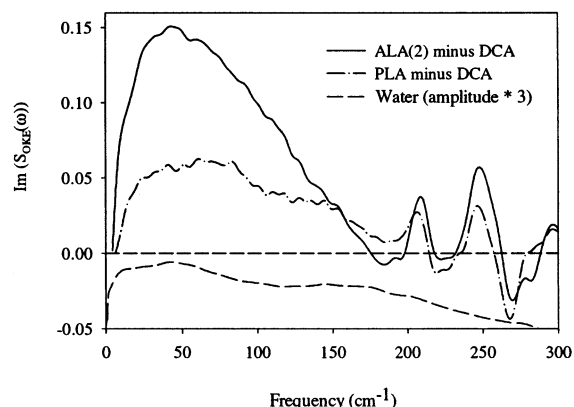


Figure 1. Low-frequency spectra taken with optical heterodyne-detected Raman-induced Kerr-effect spectroscopy (OHD-RIKES) of di-L-alanine (ALA(2)) in dichloroacetic acid (DCA), poly-L-alanine (PLA) in DCA, and water. The water spectrum has been scaled and shifted down by 0.05 for clarity.

with a shot-noise limited balanced-detection system. The ultrafast OHD-RIKES measurements were carried out using linearly polarized optical pulses centered at 800 nm. The laser beam was split into a pump beam ($\sim 80\%$) and a probe beam ($\sim 20\%$) by a beam splitter, and the pump beam was optically delayed using a 50 nm resolution stepper motor. Polarizers were placed in both arms to obtain a 45° angle between pump and probe-beam polarizations. A 6 cm focal-length lens was used to focus the two beams into the sample. Phase-sensitive detection was achieved with a chopper and lock-in amplifier. The pulse width was measured at the sample position as 20 fs fwhm ($\text{sech}^2()$) by two-photon absorption in a GaP PIN photodiode.

Balanced detection presents some advantages as compared to the traditional technique.⁷ In our setup, the probe beam is circularly polarized with a quarter-wave plate after the sample. Parallel and perpendicular components are separated with a Glan-Thompson polarizer and sent to a pair of photodiodes wired up for balanced detection.⁸ Because the signal is obtained by electronically subtracting the horizontal from the vertical component of the probe beam, it is possible to reduce the effect of random fluctuations of the laser power and achieve a significant improvement in the signal-to-noise ratio. It can be shown using a Jones-matrix analysis that by balancing with either a quarter or a half-wave plate, a signal either purely due to birefringence or purely due to dichroism is measured.

Figure 1 shows the OHD-RIKES spectra for ALA(2), PLA, and water. The spectra of the peptides have been obtained by subtraction of the pure DCA spectrum from the spectrum of the solution. The same experiment repeated with TFA showed identical spectra below 200 cm^{-1} , confirming that the signal is a measurement of the solute dynamics. The oscillatory features in the difference spectra between

* To whom correspondence should be addressed. E-mail: klaas.wynne@phys.strath.ac.uk.

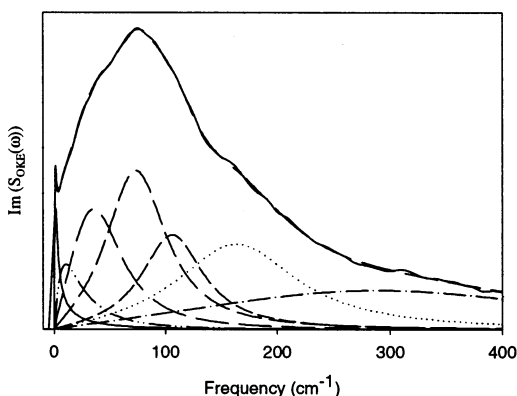


Figure 2. OHD-RIKES spectrum of an aqueous solution of lysozyme fitted with a multiple Brownian oscillator model. Modes can be detected as low as 10 cm^{-1} .

200 and 300 cm^{-1} are the result of Raman bands in DCA shifting to lower frequency on addition of peptide. These band shifts are likely due to the perturbation of hydrogen bonds through the carboxylic groups in DCA.⁹ The ALA(2) solution presents a single band similar to that seen in common organic solvents, suggesting that the small size of the dimer allows librational motion.¹⁰ In the case of PLA, the low-frequency band is more structured with two shoulders at 60 and 140 cm^{-1} . Polarized Raman and infrared spectra of oriented films of α -helical PLA¹¹ reveal shoulders at 60 and 139 cm^{-1} that have been assigned to localized backbone torsions.¹² Interestingly, those features are similar to the ones observed in the water spectrum. Previous work on water assigned the two bands at 40 and 165 cm^{-1} to transverse and longitudinal translational motions of the transient hydrogen-bond network of water.^{13–15} To explain those similarities, one can draw a parallel between the hydrogen-bond network of an α -helix and the average pentamer cluster in liquid water. The strength of a hydrogen bond increases in the presence of other hydrogen bonds in the system.¹⁶ In other words, vibrational coupling between molecules is thought to scale with the supramolecular properties of the system. Because the cooperative effect is expected to be stronger in PLA than in water, this could explain why the features around 60 and 140 cm^{-1} are more pronounced in the case of the peptide but still arise from modes similar to the translational modes in transient water clusters.

The binding to an active site or the linkage of a protein to a substrate requires flexibility and involves significant motion of the protein structure.¹⁷ The signature of such collective motions should be visible in the low-frequency Raman spectrum of a protein. Figure 2 shows the OHD-RIKES spectrum of lysozyme in aqueous solution. When converted to a normal Raman spectrum by multiplication with the Bose thermal-occupation factor,¹⁸ the spectrum is fully consistent with early studies on lysozyme and shows the earlier observed band at 75 cm^{-1} .¹⁹ In addition, the OHD-RIKES spectrum provides information hidden by the enormous intensity of the Rayleigh line in the Raman spectrum. To separate the various modes, the spectrum has been fitted with the Brownian-oscillator model¹⁸ and shows modes at 10 , 35 , 73 , 106 , 164 , and 300 cm^{-1} . Modes at 75 , 115 , and 160 cm^{-1} have been previously observed both in the crystalline form and in aqueous solution of lysozyme.¹⁹ However, a mode at 25 cm^{-1} was only detected in the solid phase. Our data confirm the presence of modes above 73 cm^{-1} and show that lower frequency modes exist even in solution. The assignment of those modes is still a matter of debate. Normal-mode

calculations indicate that modes below 40 cm^{-1} can be attributed to bending and twisting of the β -hairpin structure and those above 40 cm^{-1} to rigid-body motion of the α -helix.²⁰ However, neutron-scattering experiments on different systems suggest that librational modes of side chains interacting with water lead to spectral features observed between 10 and 30 cm^{-1} .²¹ Analytical model calculations of accordion-like motion in a 30-residue α -helix predict a mode at 25 cm^{-1} .²² Surprisingly, the spectrum in Figure 2 also shows a diffusional component corresponding to a 4.5 ps decay, consistent with diffusional motion of protein side chains. Taken together, these findings suggest that different motions occur in the very low frequency range involving probably both localized and delocalized motions.

In conclusion, our results show a clear conformational dependence in the low-frequency spectra between the single band centered at 50 cm^{-1} of ALA(2) and the two shoulders at 60 and 140 cm^{-1} of the α -helical PLA. The similar spectral features observed in PLA and water could be explained by analogous acoustic translational modes through the hydrogen-bond network of the PLA α -helix. Because the peptides are in solution, the modes observed are damped, which tends to localize the excitations. It would be interesting to compare our results with crystalline PLA in which the damping of delocalized modes would be much reduced. The spectrum of lysozyme in solution confirmed the presence of modes at 73 , 106 , and 164 cm^{-1} and showed additional modes at very low frequency, 10 and 35 cm^{-1} , showing that low-frequency delocalized modes in proteins persist in solution.

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