Dynamical Test of Davydov-Type Solitons in Acetanilide Using a Picosecond Free-Electron Laser

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Picosecond-infrared-excitation experiments on acetanilide, an α-helix protein analog, indicate that the anomalous 1650-cm^{-1} band which appears on cooling of acetanilide crystals persists for at least several microseconds following rapid pulsed heating. The ground-state recovery time is 15 ± 5 ps, consistent with a conventional mode strongly coupled to the phonon bath. We therefore suggest that the unusual temperature-dependent spectroscopy of acetanilide can be accounted for by slightly nondegenerate hydrogen-atom configurations in the crystal.

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The mechanism of energy transport in proteins has been an active area of research for over 20 years.[1] Our lack of a satisfactory picture of how the energy required for protein function and chemistry travels over considerable molecular dimensions without being thermally dissipated is a fundamental gap in our understanding of protein function. As a remedy, Davydov proposed that self-trapped vibrational excitations in the peptide groups linking the helices could propagate along the chains and transport energy.[2] An exciting and controversial development in the experimental search for Davydov-type solitons was the report of Careri *et al.*[3] who argued that solitons could be photogenerated by infrared excitation of the "unconventional" amide-I absorption band of the amino-acid analog acetanilide (ACN) at 1650 cm^{-1}. Acetanilide crystallizes into a structure containing chains of hydrogen-bonded amide groups which make it a simple analog to α-helix proteins for the study of energy transport.[4]

The rationale for the assignment of the 1650-cm^{-1} band is manifold:[5] First, it is an "extra"[6] vibrational band which appears in the crystal but not the amorphous material. Second, the band has an unusual temperature dependence featuring a dramatic increase in intensity on cooling. This intensity dependence can be fitted by a modified soliton model where the oscillator strength of the soliton creation is enhanced by interaction with the ground-state population of a 130-cm^{-1} crystal phonon. Moreover, it was argued that no change in structure is observed in either x-ray or specific-heat data, making structural changes[7,8] an unlikely explanation. Lack of sample-to-sample variation suggested that the anomalous band was an intrinsic effect.[9] There remains considerable controversy as to the nature of these ir excitations in ACN and whether they bear upon the biophysics of α-helix proteins. Several recent dynamical simulations of Davydov solitons[10-12] indicate that the Davydov solitons, if they exist, would have too short a lifetime at room temperature (subpicosecond) to be useful for energy transport or storage.

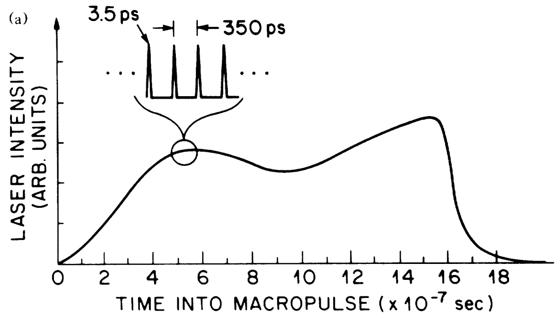
Unfortunately most of the debate has been based either on theoretical work or on static spectroscopy. Very little experimental work on the dynamics has been done to help to resolve the issue.[13] Therefore, we set out to measure the relaxation time for the vibrational excitation of the 1650-cm^{-1} band since the lifetime and the mobility of the soliton are important criteria for its utility as a means of energy storage or transport. In particular, if the excitation is self-trapped then its interaction with the lattice phonon bath would be weak and one might expect such solitonlike excitations to be relatively long lived, on the order of hundreds of picoseconds.[14]

The most straightforward way to set limits on the excitation lifetime is by transient-infrared-bleaching experiments. The source required for these must be tunable around 6 μm (1650 cm^{-1}) with adequate spectral resolution to selectively excite the band (10 cm^{-1}). In addition, pulses with picosecond duration and sufficient intensity to bleach the transition are necessary to observe the bleaching recovery with adequate temporal resolution and signal-to-noise ratio.

The source we have chosen to meet these criteria is the Mark III free-electron laser[15] (FEL) and its salient properties are enumerated below. In the present paper, we describe transient-bleaching-recovery experiments on the 1650-cm^{-1} band of ACN. These are the first experiments which exploit the picosecond time resolution of a FEL. We measure rapid (≤ 20 ps) bleaching recovery of the 1650-cm^{-1} band and we show that its behavior under rapid heating is unexpectedly stable, supporting the idea that structural rearrangements are the explanation for the properties of the unconventional amide-I band in ACN.

The basic operating principles and specifications of the Mark III FEL have been reviewed elsewhere.[15] Here, we only document several key features of the laser important for understanding our experiments. The laser pulses come in bursts ("macropulses") about 1.3 μs in duration as shown in Fig. 1(a). The inset shows the substructure consisting of nearly transform-limited, 3.5-ps "micropulses" separated by 350 ps. There are sixteen macropulses per second of 2–3 mJ each at 1650 cm^{-1}. The laser's frequency is continuously tunable and has about 7 cm^{-1} bandwidth at any given time during the macropulse. However, as we show in Fig. 1(b), the optical frequency of the micropulse "chirps" from high frequency to low frequency during the macropulse. The size of this chirp is about 20 cm^{-1} across the macropulse. Thus, at a fixed delay of some number of picoseconds between pulse and the probe there is a scan of approximately 20 cm^{-1} during the macropulse. Relaxation of the transmission as a function of both time and wavelength can be measured by averaging of the macropulse signal as a function of pump-probe delay. One aspect of this pulse structure is that significant sample heating is unavoidable during the macropulse and we will discuss its manifestations in detail.

The Mark III laser output is divided into pump (95%) and probe (5%) beams by a wedged CaF_2 plate. The beam lines are purged with N_2 for most of their length to reduce atmospheric H_2O absorption. The pump beam undergoes a variable-path-length delay and the optics are arranged so that pump and probe travel parallel paths towards the sample. Both pulses are focused by a single lens to a 300-μm-diam spot on the sample which is held in a closed-cycle refrigerator with CaF_2 windows. The spot diameter was the minimum diameter for which no sample damage was observed. Part of the probe is split off to be used as a reference and is observed by a liquid-nitrogen-cooled AuGe detector. The probe transmission through the sample is monitored by a second AuGe detector and both of these signals are recorded as in Fig. 1(a) by a dual-trace digital 100-MHz oscilloscope.



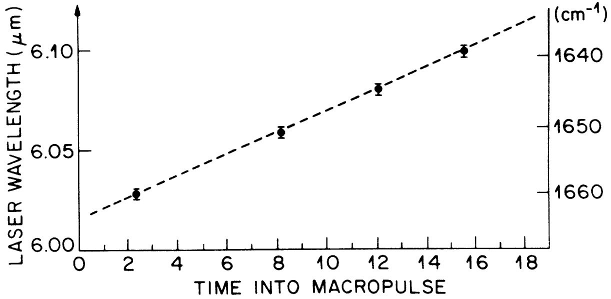


FIG. 1. Optical properties of the Mark III free-electron laser. (a) Temporal shape of the macropulse as viewed by the reference AuGe detector. Inset: The picosecond microstructure of the pulses. (b) Frequency chirp measured during the macropulse.

The ACN was purified by repeated sublimation. Crystalline domains were grown by slow cooling from an ACN melt between CaF_2 disks of 2 mm thickness. The Fourier-transform infrared (FTIR) spectra of the samples are in agreement with the literature spectra of crystalline ACN. After our experiments the FTIR spectrum of the pumped region of the sample was unchanged. Based on its infrared absorption spectrum, the sample is about 5000 Å thick. Pump scattering into the probe detector was relatively weak and was subtracted experimentally.

Figure 2(a) displays the normalized transmission versus time into the macropulse, t_M. The large decrease in transmission observed upon sample insertion is due to both reflection and scattering losses in the sample and substrate. FTIR spectra indicate that these account for half of the transmission loss. As expected, the trace without the sample is flat while the 250-K spectrum has a small transmission dip at the energy corresponding to the 1650-cm^{-1} amide-I band. This dip increases by about a factor of 2 on cooling from 250 to 80 K in qualitative agreement with the FTIR spectra of Careri *et al.*[3] This confirms that we are probing the anomalous amide-I band.

A substantial increase in the probe-pulse transmission at the 1650-cm^{-1} band was observed when the pump pulse was simultaneous with the probe pulse. The fractional change in transmission $\Delta I/I$ caused by the pump beam is plotted versus time in the macropulse t_M in Fig. 2(b) for several time delays between the pump and probe pulses. The transmission increase due to the pump roughly follows the 1650-cm^{-1} band shape at 0-ps delay (pump and probe temporally overlapped), indicating that the effect is associated with the 1650-cm^{-1} band.

Figure 3 shows the bleaching recovery dynamics at the band center as assembled from many scans like those of Fig. 2(b). The fast component in Fig. 3 is pulse-duration limited and we believe it to be largely due to degenerate four-wave mixing which is automatically phase matched by our experimental geometry as long as the pump and probe pulses remain temporally overlapped.[16] The slow component has a decay time of 15 ± 5 ps and we associate it with saturation recovery due to repopulation of the ground state of the 1650-cm^{-1} mode. Evidence that these components correspond to different processes comes from data like those of Fig. 2(b) where the spectral peaks of the bleaching for delays ≤ 2 ps and delays

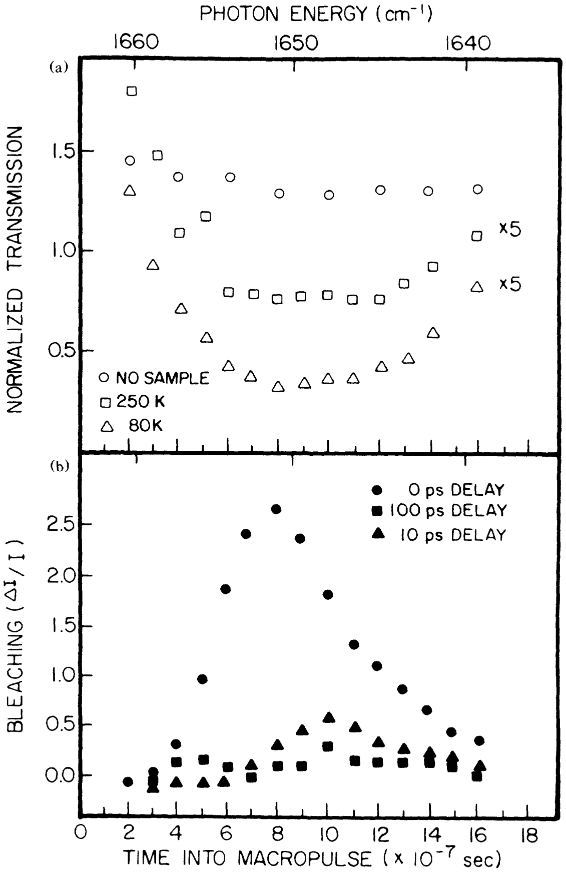


FIG. 2. (a) Transmission of the ACN sample without pump excitation. The probe transmission is divided by a portion of the laser which does not go through the sample. The resulting normalization corrects for laser-energy fluctuations. Note that the macropulse time has been converted to wave number on the top axis. (b) The fractional change in normalized transmission due to the presence of a pump beam. The data are corrected for the presence of a small amount of pump scattering into the detector. The ambient temperature was 80 K.

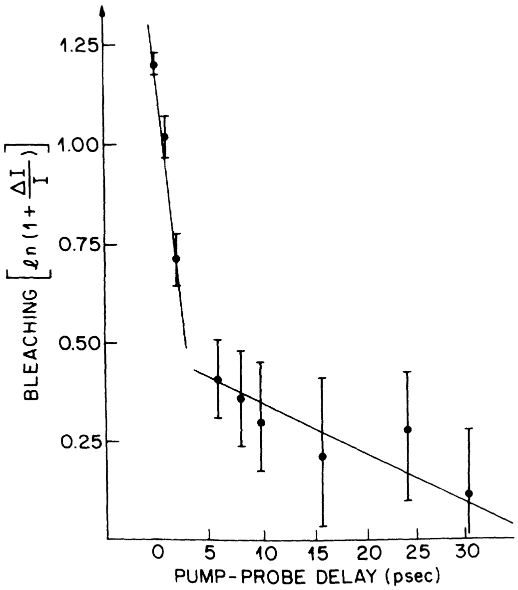


FIG. 3. Probe bleaching vs pump-probe delay at 1651 cm^{-1} and 80-K ambient temperature.

≥ 5 ps are displaced and also from the observation that the signal for ≤ 2-ps delay is superlinear with pump energy. Extrapolation of the slow component back to zero delay gives $\Delta I/I$ due to saturation of about 0.4. Since $\Delta I/I = e^{\Delta N\sigma l} - 1$, where ΔN is the number of bleached amide-I modes per volume and σ is their cross section, we can calculate the expected bleaching for 10 J/cm^3 (4×10^{20} photons/cm^3) absorbed by assuming that one photon bleaches one amide-I mode. We know σl from the absorption of the sample, $I_{\text{out}}/I_0 = e^{-N\sigma l}$, where $N = 4\times10^{21}$/cm^3 is the molecular density. Therefore, the expected bleaching for our pump energies is about 0.1, of comparable magnitude. The relaxation time of 15 ps indicates that the 1650-cm^{-1} band is strongly coupled to the lattice and has typical relaxation values for a vibrational line in a molecular crystal.[17]

We now consider the implications of the transmission recovery of the sample at 100-ps probe delay to the "cold" base line. This is an important fact since the 1650-cm^{-1} band should disappear on deposition of energy into the 130-cm^{-1} optical phonons in the case of soliton theory.[8] Loss of the 1650-cm^{-1} band due to such heating should result in approximately a factor-of-2 transmission increase at 100 ps, which is not observed. Since the time scale of such vibrational relaxation is on the order of 10^{-10} s,[13] our data are therefore not consistent with the soliton hypothesis, unless the rate of energy flow into the 130-cm^{-1} phonon is enormously slower than in other materials.

Simple thermal-conductivity calculations using the thermal conductivity of H-bonded crystals[18] show that the 16-Hz repetition rate of the FEL macropulses is low enough that the sample should cool to ambient temperature between macropulses. During the macropulse, however, a substantial temperature rise occurs. A single incident 2-ps micropulse deposits approximately 10 J/cm^3 leading to about a 10-K heating. If we assume the quasistatic heating data of Fig. 2(a) or Ref. 3 to apply, then the maximum temperature rise consistent with the less than 15% bleaching we observe at 100-ps delay is only 30 K. Thus, our 0.5-μm-thick sample would have to cool in less than the time for three micropulses (less than 1 ns). We are quite confident that this is impossibly rapid cooling. For example, consider that the time for an acoustic wave to traverse the 0.5-μm sample is already almost 1 ns. Thermal diffusion and heat flow are obviously much slower than this.

The above argument is very loose but is free of assumptions about heat flow and boundary conditions. A more proper method to estimate the temperature rise is to calculate the temperature when heat flow out of the excitation region equals the rate of energy deposited by th laser. The result of such a calculation is a temperature rise of 150 K even when we assume that the substrate remains cold. This sets a lower limit on the actual temperature rise which is probably much higher. In fact, we wish to address the possibility that the macropulse energy is sufficient to melt the sample. We can rule this out based upon two observations: (1) The amide-I region indicates large absorption shifts upon melting[19] which would show up as a large change in the absorption of the sample at 1650 cm^{-1}. Since the absorbance at 100-ps delay does not change due to the pump, it is very unlikely that the sample is melted. (2) Visible surface damage to materials under pulsed-laser irradiation is usually correlated with melting of the surface. Since the spot size was adjusted so that no visible surface damage to the ACN crystal occurred, this also speaks against sample melting during the macropulse.

The energy deposited per unit volume per micropulse is about 10 $\mathrm{J/cm}^3$, or about 5-K heating from the measured specific heat.[3] It should take about twenty micropulses (6 ns) for the sample to reach the equilibrium temperature of 230 K (80+150) estimated above during the macropulse, and we can therefore consider the sample to be at the elevated temperature through the entire macropulse. The lack of bleaching at 1650 cm^{-1} observed at 100-ps delay after the pump [Fig. 2(b)] is not consistent with the equilibrium temperature rise of 150 K.

The inescapable conclusion is that the ACN samples behave differently under rapid (μs) pulsed heating than under slow heating as in the experiments of Ref. 3. The fact that the 1650-cm^{-1} band persists in the pulsed-heating case suggests that it is not due to a self-trapped vibrational state but perhaps is due to multiple configurations for the amide group which are separated by a thermal barrier. Thus, the absence of bleaching at large pump-probe delays could occur if there are stable nondegenerate configurations with energy difference Δ and if the relaxation rate between configurations is longer than several microseconds at a given temperature. It is likely that the major change is in the position of the hydrogen-bonding proton and that x-ray diffraction would not be sensitive to this. Double-well descriptions of the proton position in hydrogen-bonded systems are common.[20] For concreteness, we take two wells associated with the 1650- and 1665-cm^{-1} amide-I modes in ACN. The simplest rate of equilibration between them is given by an Arrhenius form $R_{ab}=R_0e^{-E/kT}$. We assume that the relaxation rate between the wells must be less than $10^6\ \mathrm{s}^{-1}$ at approximately 300 K from this experiment, and from the low-temperature spectroscopy it is observed that the equilibration rate is much less than 0.1 s^{-1} at 77 K. Self-consistency of these rates with the Arrhenius law gives a value for the barrier E of approximately 1000 cm^{-1} and a preexponential of approximately $10^8\ \mathrm{s}^{-1}$. Such values are observed when there is substantial nuclear motion[21] and are thus not implausible.

Although this Letter casts strong doubt upon the soliton model of energy transmission in polypeptides, the technique described here of pump-probe spectroscopy in the ir on a picosecond time scale should prove useful for testing the actual means by which energy flow does occur in biomolecules, and in this sense the results presented here are very encouraging.

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