Direct Observation of Self-Trapped Vibrational States in α-Helices

J. Edler,[1] R. Pfister,[1] V. Pouthier,[2] C. Falvo,[2] and P. Hamm[1]

[1]*Physikalisch-Chemisches Institut, Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland*

[2]*Laboratoire de Physique Moléculaire, UMR CNRS 6624, Faculté des Sciences, La Boúloie, Université de Franche-Comté, 25030 Besançon Cedex, France*

(Received 13 May 2004; published 3 September 2004)

Femtosecond infrared pump-probe spectroscopy of the N-H mode of a stable α-helix reveals two excited-state absorption bands, which disappear upon unfolding of the helix. A quantitative comparison with polaron theory shows that these two bands reflect two types of two-vibron bound states connected to the trapping of two vibrons at the same site and at nearest neighbor sites, respectively. The latter states originate from an acoustic phonon in the helix, which correlates adjacent sites.

DOI: 10.1103/PhysRevLett.93.106405 PACS numbers: 71.38.Ht, 63.20.Ry, 63.22.+m, 82.53.Ps

The formation of self-trapped states, or phonons, caused by the interaction of excitons with lattice vibrations, can result in exciting energy transport properties and novel optical characteristics. Self-trapping has been observed in various materials. In particular, quasi-one-dimensional structures, such as halogen-bridged metal complexes [1,2] or hydrogen bonded molecular crystals [3–10], are ideal systems to study self-trapping. In the first case, the excitations are electronic, while they are vibrational in the second case. It has been suggested that vibrational self-trapping in α-helices plays an important role in the remarkable efficiency of energy transport and energy storage in proteins [11].

An α-helix consists of a helical sequence of amino acids, which is stabilized by three quasi-one-dimensional chains of hydrogen bonds. According to Davydov's model [11], individual molecular oscillators of the peptide unit (-CONH-), such as the N-H or C═O stretching mode, are coupled through electrostatic interactions, leading to a delocalization of the excitation, i.e., a vibrational exciton or vibron. The vibron, in turn, is coupled to lattice phonons along the hydrogen bonded chain through an anharmonic (nonlinear) term, which is mediated by the highly nonlinear hydrogen bonds. Vibrational self-trapping has been investigated in detail for hydrogen bonded molecular crystals, such as crystalline acetanilide (CH_3-CONH-C_6H_5, ACN) [3–10]. These crystals are considered to be model systems for α-helices with similar local structures of the peptide unit and the hydrogen bonds. The infrared absorption spectrum of ACN shows temperature dependent anomalies that have been assigned to vibrational self-trapping [4,10]. Edler and Hamm have recently confirmed this interpretation by employing infrared femtosecond pump-probe spectroscopy [12–15].

However, compelling experimental evidence for self-trapping in real α-helices or proteins is extremely rare. The only work we are aware of is that of Ref. [16], which observed a lengthened lifetime of certain vibrational states in myoglobin, and Ref. [17], which observed an anomalous temperature dependence in the N-H spectrum of polyalanine. Here, we monitor self-trapped states directly through their anharmonicity. To study anharmonicity, the method of choice is femtosecond vibrational spectroscopy, since the nonlinear response of a completely harmonic system vanishes identically [18].

We chose poly-γ-benzyl-L-glutamate (PBLG) for this investigation, because it forms extremely stable, long α-helices in both helicogenic solvents and films grown from these solvents. The monomeric unit of PBLG is a non-natural amino acid with a long side chain that stabilizes the helix; however, the helix backbone is identical to that of natural helices. Because of its stability, PBLG has served as the standard model helix since the very early days of structural investigations of proteins [19]. PBLG with a chain length of $\approx$90 amino acids was either dissolved in chloroform (concentration $\approx$0.5–1 mM) or used as films grown from such a solution.

Figure 1(a) (red line) shows the absorption spectrum of the helix in chloroform solution at 293 K (with the solvent spectrum subtracted), which is dominated by the strong N-H stretching band at 3290 cm^{-1}. Figure 1(b) (red line) shows the pump-probe response of the helix 600 fs after

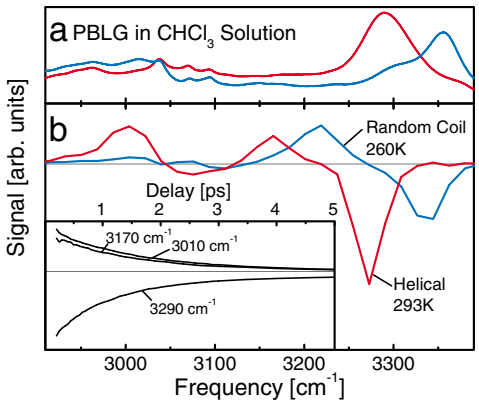

FIG. 1 (color). (a) Absorption spectra of PBLG in chloroform with 3% TFA at 293 K (red line, helical conformation), and at 260 K (blue line, random coil). (b) Pump-probe spectra 600 fs after excitation under the same conditions. Inset: Decay of negative and both positive bands at 293 K.

excitation with an ultrashort broadband excitation pulse (duration 150 fs, bandwidth 200 cm^{-1}, energy density $\approx$10 mJ/cm^2). We find a negative band at 3280 cm^{-1} [20] and two positive bands at 3160 and 3005 cm^{-1}, respectively. The two positive peaks are very characteristic for the helix and appear under almost any condition (see below).

The experiment in Fig. 1 has been performed with a little additive (3%) of trifluoroacetic acid (TFA) as denaturant. At this concentration, the helix is folded and stable at room temperature, but unfolds to a random coil with a sharp transition at about $-5\,^{\circ}C$ (cold denaturation) [21]. Figure 1(a) (blue line) shows the absorption spectrum of a random coil ensemble at $-18\,^{\circ}C$, where the N-H band blueshifts to 3355 cm^{-1}. In an intact helix, the N-H-groups form *intramolecular* hydrogen bonds with C═O groups from the next helix turn, while they form *intermolecular* hydrogen bonds with TFA molecules in a random coil configuration (which is why TFA destabilizes the helix). The latter are weaker hydrogen bonds, explaining the blueshift of the N-H band. The negative band in the pump-probe experiment [Fig. 1(b), blue line] shifts accordingly. The important difference, however, is that only *one* positive band is observed in the random coil configuration.

If the N-H stretching vibrators were isolated—isolated from other peptide units in the α-helix as well as from other normal modes within one peptide unit—one would expect the common pump-probe response of anharmonic oscillators [18]: a negative band at the frequency of the N-H fundamental associated with bleach ($\nu = 0 \rightarrow \nu = 1$) and stimulated emission ($\nu = 1 \rightarrow \nu = 0$), and a positive band associated with excited-state absorption ($\nu = 1 \rightarrow \nu = 2$). The excited-state absorption band is expected to be redshifted with respect to the bleach and stimulated emission signal due to the anharmonicity of the vibrator. This kind of pump-probe response is very characteristic and is always observed when isolated molecules are vibrationally excited (see, e.g., Ref. [22] and many other examples). When the molecule is unfolded, the individual N-H groups, indeed, appear to be isolated, and we obtain exactly the expected response with an anharmonicity of $\omega_{01} - \omega_{12} \approx 120\ cm^{-1}$. In contrast, the observation of *two* positive bands for the intact helix [Fig. 1(b), red line], rather than just one, is *exceptional* and is the major topic of this Letter.

We have performed numerous test experiments to assign the double-peak spectral signature.

Temperature dependence.—In a film, the helix does not unfold even at temperatures down to 18 K. In that case, the double-peak structure stays the same over the whole temperature range from 18 to 293 K [Fig. 2(b)]. This finding proves that the spectral change in Fig. 1(b) is not a direct temperature effect, but indirect through a temperature induced structural change of the helix.

Polarization dependence.—The anisotropies of both positive bands are identical and agree with that of the bleach–stimulated emission signal [Fig. 2(c)]. Hence, the transition dipoles of all signals are parallel.

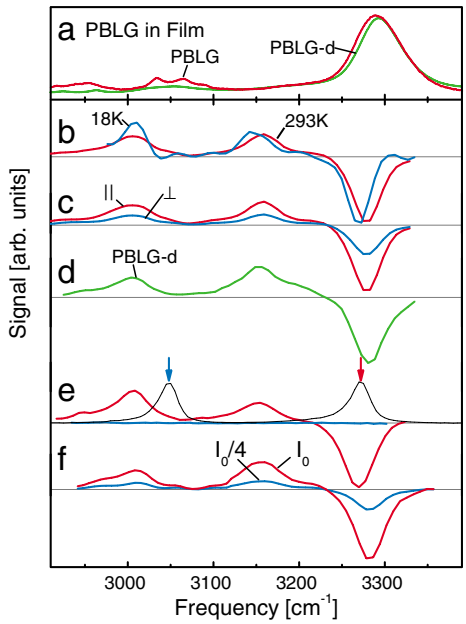

FIG. 2 (color). (a) Absorption spectra of PBLG (red) and fully C-deuterated PBLG (green) in films grown from chloroform solution. (b) Pump-probe response at 293 K (red) and 18 K (blue). (c) Polarization dependence of pump-probe response. (d) Pump-probe response of fully C-deuterated PBLG. (e) Pump-probe response of PBLG-d upon narrow band excitation at 3272 cm^{-1} (red) and 3047 cm^{-1} (blue). The pump-pulse spectra are shown as black lines. (f) Pump-probe response with the pump-pulse intensity varied by a factor of 4.

Time dependence.—When varying the pump-probe delay time, both the positive and the negative bands decay in parallel with a 1.5 ps time constant [Fig. 1(b), inset]. This indicates that all these bands originate from one and the same state, and disappear simultaneously as this state decays.

Fully C-deuterated PBLG.—The absorption spectrum exhibits small bands between 2900 and 3100 cm^{-1} [Fig. 2(a), red line], which are mostly due to CH stretching vibrations. They can be eliminated when C-deuterating the helix [Fig. 2(a), green line]. Fully C-deuterated PBLG (PBLG-d) has been synthesized as described in Refs. [23,24]. The pump-probe response of PBLG-d [Fig. 2(d)] reveals the same double-peak structure as PBLG, showing that the CH bands do not contribute.

Narrow band excitation.—When eliminating the CH stretching vibrations, a further small band remains, which is assigned to a weak overtone or combination mode. In order to assure that the overtone does not contribute, we performed frequency selective pump-probe experiments with a spectrally filtered pump pulse [using an adjustable Fabry-Perot filter with bandwidth 30 cm^{-1}, the pump-pulse spectra are shown in Fig. 2(e) as black lines]. The result shows that the double-peak

structure appears only when pumping the N-H band, while no measurable signal is obtained when pumping the overtone.

Intensity dependence.—Multiphoton excitation has been excluded by reducing the pump-pulse intensity by a factor of 4 [Fig. 2(f)], still revealing the same double-peak structure.

Fermi resonance.—It has been argued that the N-H band of proteins, peptides, and peptide model systems is complicated by a Fermi resonance with the amide II mode at 1550 cm^{-1} [25]. The Fermi resonance is usually weak in absorption spectroscopy because of the large frequency mismatch ($2\omega_{\text{amide II}} - \omega_{\text{NH}} = -190\ cm^{-1}$). Accordingly, only a very weak band is found at about 3050 cm^{-1} [Fig. 2(a), green line], which could be the amide II overtone. However, owing to the much larger intrinsic anharmonicity of the N-H band, the resonance condition improves for the excited-state absorption bands. Hence, the double-peak structure in the pump-probe spectra could reflect, for example, a Fermi resonance between the first overtone of N-H mode ($2\nu_{\text{NH}}$) and the $\nu_{\text{NH}} + 2\nu_{\text{amide II}}$ combination mode. The Fermi resonance would be almost symmetric, since the intensities of the two bands are comparable, and thus would have to be exceptionally strong, as judged from the large splitting of 155 cm^{-1}. Upon unfolding, the N-H band blueshifts by 65 cm^{-1}, while the amide II band remains at its original frequency. Given the large Fermi splitting of 155 cm^{-1}, a detuning of the resonance by 65 cm^{-1} clearly is not sufficient to explain a complete disappearance of the Fermi resonance. We have furthermore measured the anharmonic coupling terms by performing two-color pump-probe experiments, where the N-H band is excited and the spectral range of the amide I and the amide II is probed. The results are entirely inconsistent with a Fermi splitting of the order of 155 cm^{-1}. Taking these arguments together, we can safely exclude a Fermi resonance as the possible explanation for the double-peak signature in the pump-probe response.

To summarize the experimental part, we conclude that the two positive bands observed in the pump-probe response (i) originate from the N-H band solely and (ii) are associated with a coupling mechanism *between* peptide units, and not *within* individual peptide units. The two bands represent transitions to two-vibron states, and require an intact helix structure. Hence, when looking for an explanation of this spectral signature, we may restrict our search to the N-H modes, to intersite degrees of freedoms that exist only in a regular structure, and to couplings between the N-H modes and these intersite degrees of freedoms.

In this context, Pouthier and co-workers have recently investigated the two-vibron dynamics in α-helices with special emphasis onto the interplay between the intramolecular anharmonicity and strong vibron-phonon coupling [26,27]. The result of this work is briefly reviewed in the following: According to Davydov's model [11], the system is described as a 1D chain formed by N sites periodically distributed along the lattice. Each site is occupied by a peptide group which contains the N-H stretching vibration. The latter behaves as a high frequency anharmonic oscillator coupled to acoustic phonons, which characterize the external dynamics of the peptide groups [28]. The vibrational excitations reduce to anharmonic vibrons dressed by a virtual cloud of phonons, i.e., anharmonic small polarons. Both the intramolecular anharmonicity and the dressing effect favor the formation of two-vibron bound states which correspond to the trapping of two quanta over only a few neighboring sites with an energy that is smaller than the energy of two quanta lying far apart. The two-vibron energy spectrum exhibits three types of states (see Fig. 3, inset): (i) two-vibron free states (TVFS) belonging to an energy continuum which correspond to two noninteracting vibrons, (ii) two-vibron bound states I (TVBS-I) which refer to the trapping of the vibrons at the same site, and (iii) two-vibron bound states II (TVBS-II) which characterize vibrons trapped at nearest neighbor sites. The occurrence of TVBS-II, which originates from the overlap between the virtual clouds of phonons of each vibron, appears as a signature of the acoustic nature of the phonons responsible for correlations between adjacent sites. TVBS-I and TVBS-II form two energy bands located below the TVFS continuum.

Compared with the previous work [26,27], which was devoted to the amide I vibrations, some modifications have to be made to account for the stronger nonlinearity of the N-H modes. First, the N-H polaron hopping constant almost vanishes due to the dressing effect so that the polaron mass becomes infinite. Therefore, the TVFS bandwidth, as well as the dispersion of both bound state bands, are negligible. Second, in addition to the Davydov coupling $\Delta H_{vp} = \sum_n \chi(u_{n+1} - u_{n-1}) b_n^\dagger b_n$ (where u_n refers to the phonon displacement and b_n and $b_n^\dagger$ stand for the vibron operators), a state-dependent coupling has been introduced via an additional term $\Delta H'_{vp} = -\sum_n \chi'(u_{n+1} - u_{n-1}) b_n^{\dagger 2} b_n^2$.

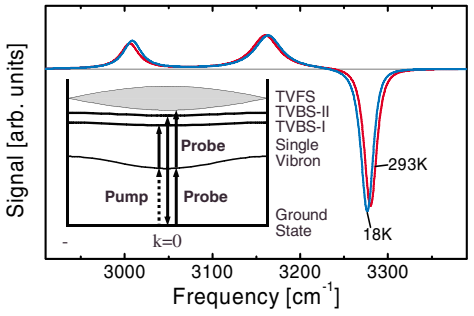

FIG. 3 (color). Simulated pump-probe spectrum for 293 K (red line) and 18 K (blue line) using the theory of Ref. [26]; for parameters see text. Inset: Schematic of the energy levels (not on scale).

With these modifications, the theory detailed in Refs. [26,27] is used to compute the pump-probe signal, using the following parameters: harmonic N-H frequency $\omega_0 = 3520\ \mathrm{cm}^{-1}$, phonon cutoff frequency $\Omega_c = 100\ \mathrm{cm}^{-1}$ [7], intramolecular anharmonicity $A = 60.0\ \mathrm{cm}^{-1}$ (i.e., the anharmonicity of isolated N-H groups in the unfolded helix), undressed hopping constant $J = 5\ \mathrm{cm}^{-1}$, i.e., a typical value for vibron hopping in proteins (see, for instance, Ref. [10]), and small polaron binding energy $E_B = 84\ \mathrm{cm}^{-1}$ (see, for instance, Ref. [7]). The ratio χ'/χ is fitted to 0.22. Finally, an arbitrary linewidth is used, which takes into account that the dephasing rate of TVBS-I is 1.5 times smaller than that of TVBS-II [27].

Figure 3 clearly shows that the model reproduces the experimental pump-probe spectra (Figs. 1 and 2) very well. At $T = 293\ \mathrm{K}$, it reveals a single negative peak located at $3280\ \mathrm{cm}^{-1}$ and two positive peaks located at 3005 and $3160\ \mathrm{cm}^{-1}$, respectively. The negative peak refers to the bleach and stimulated emission between the ground state and the zero wave vector single-vibron state. This process overcompensates the absorption from the single-vibron state to the zero wave vector TVFS, which takes place at the same frequency. In contrast, the positive peaks located at 3005 and $3160\ \mathrm{cm}^{-1}$ correspond to the excited-state absorption from the zero wave vector single-vibron state to the zero wave vector TVBS-I and TVBS-II, respectively. From the theory we expect for the integrated intensities $I_{\mathrm{TVBS\text{-}I}}/I_{\mathrm{TVBS\text{-}II}}/I_{\mathrm{TVFS}} \approx 1/2/-3$, in good agreement with the experiment [e.g., Fig. 2(d)]. Note that these intensities are inherent to the model, and do not contain explicit fit parameters. Furthermore, we find that the theoretical spectrum is almost temperature independent as a result of the infinite polaron mass due to the strong dressing effect (Fig. 3), again reproducing the experimental finding [Fig. 2(b)].

In conclusion, the experimental results unambiguously show that the two positive bands are a signature of the helical conformation, in which individual N-H vibrations are correlated by acoustic phonons. The phonons enable the formation of self-trapped states that can be identified through their anharmonicity. When the helical structure is destroyed, the correlation vanishes and the experimentally observed pump-probe response is that of an isolated vibrator. The theory assigns the two positive peaks to the existence of two kinds of two-vibron bound states correspond to the trapping of two vibrons at the same site and at nearest neighbor sites, respectively. The latter states originate from the overlap between a virtual cloud of acoustic phonons with each vibron. To our knowledge, this is the first direct observation of vibrationally self-trapped states in α-helices, some 30 years after their prediction by Davydov [11].

The work has been supported by the Swiss National Science Foundation (2100-067573/1).

[1] B. I. Swanson, J. A. Brozik, S. P. Love, G. F. Strouse, A. P. Shreve, A. R. Bishop, W.-Z. Wang, and M. I. Salkola, Phys. Rev. Lett. **82**, 3288 (1999).
[2] S. L. Dexheimer, A. D. V. Pelt, J. A. Brozik, and B. I. Swanson, Phys. Rev. Lett. **84**, 4425 (2000).
[3] G. Careri, U. Buontempo, F. Carta, E. Gratton, and A. C. Scott, Phys. Rev. Lett. **51**, 304 (1983).
[4] G. Careri, U. Buontempo, F. Galluzzi, A. C. Scott, E. Gratton, and E. Shyamsunder, Phys. Rev. B **30**, 4689 (1984).
[5] G. B. Blanchet and C. R. Fincher, Phys. Rev. Lett. **54**, 1310 (1984).
[6] D. M. Alexander, Phys. Rev. Lett. **54**, 138 (1985).
[7] D. M. Alexander and J. A. Krumhansl, Phys. Rev. B **33**, 7172 (1986).
[8] W. Fann, L. Rothberg, M. Roberson, S. Benson, J. Madey, S. Etemad, and R. Austin, Phys. Rev. Lett. **64**, 607 (1990).
[9] S. W. Johnson, M. Barthes, J. Eckert, R. K. McMullan, and M. Muller, Phys. Rev. Lett. **74**, 2844 (1995).
[10] A. C. Scott, Phys. Rep. **217**, 1 (1992).
[11] A. S. Davydov, J. Theor. Biol. **66**, 379 (1977).
[12] J. Edler, P. Hamm, and A. C. Scott, Phys. Rev. Lett. **88**, 067403 (2002).
[13] J. Edler and P. Hamm, J. Chem. Phys. **117**, 2415 (2002).
[14] J. Edler and P. Hamm, J. Chem. Phys. **119**, 2709 (2003).
[15] J. Edler and P. Hamm, Phys. Rev. B **69**, 214301 (2004).
[16] A. Xie, L. van der Meer, W. Hoff, and R. H. Austin, Phys. Rev. Lett. **84**, 5435 (2000).
[17] V. Helenius, J. Korppi-Tommola, S. Kotila, J. Nieminen, R. Lohikoski, and J. Timonen, Chem. Phys. Lett. **280**, 325 (1997).
[18] P. Hamm and J. Edler, in *Energy Localisation and Transfer*, edited by T. Dauxois, A. Litvak-Hinenzon, R. MacKay, and A. Spanoudaki (World Scientific, Singapore, 2004), pp. 301–324.
[19] P. Doty, J. H. Bradbury, and A. M. Holtzer, J. Am. Chem. Soc. **78**, 947 (1956).
[20] We find a systematic shift of $\approx -10\ \mathrm{cm}^{-1}$ between absorption band and bleach signal. This shift is attributed to disorder broadening, where the more delocalized states with higher dipole strength located at the low frequency side of the spectrum contribute stronger to the nonlinear response.
[21] J. A. Ferretti and B. W. Ninham, Macromolecules **3**, 30 (1970), and references therein.
[22] P. Hamm, M. Lim, and R. M. Hochstrasser, J. Chem. Phys. **107**, 10 523 (1997).
[23] E. R. Blout and R. H. Karlson, J. Am. Chem. Soc. **78**, 941 (1956).
[24] W. H. Daly and D. Poché, Tetrahedron Lett. **29**, 5859 (1988).
[25] S. Krimm and J. Bandekar, Adv. Protein Chem. **38**, 181 (1986).
[26] V. Pouthier, Phys. Rev. E **68**, 021909 (2003).
[27] V. Pouthier and C. Falvo, Phys. Rev. E **69**, 041906 (2004).
[28] The absence of optical phonons in the α-helix may explain the absence of side peaks in the N-H absorption spectrum [10] that are typically observed in molecular crystals such as ACN.