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Temperature Dependence of Low-Frequency Coherent Vibrational Motions in Bacterial Reaction Centers

Abstract

We report on the temperature dependence of the oscillatory features in the near-infrared emission band induced by direct excitation in the dimer absorption band of a bacterial reaction center.

Introduction

A major achievement allowed by the development of femtosecond spectroscopic techniques has been the real time vizualization of nuclear motion during the course of a chemical reaction (Khundkar and Zewail, 1990). In these studies, a wave packet is created on the potential energy surface of an electronic state by impulsive perturbation of an ensemble of molecules by a short light pulse and the dynamics of such a packet is subsequently inspected by a delayed probe pulse. The pioneering studies in this field were performed with simple alkali molecules in the gas phase, where dephasing mechanisms by intra- and intermolecular energy exchange between vibrational modes is virtually absent on the picosecond time scale. Extension of these studies to simple molecules in liquids has allowed to study the dephasing process by the solvent bath.

Oscillatory features have also been observed in the hexameric chromophore complex bound to the bacterial reaction center, a very complex protein macromolecule (Vos et al., 1991). We have recently demonstrated the vibrational origin of these features (Vos et al., 1993). The fact that specific frequencies ($< 100\ cm^{-1}$) dominate the oscillations in femtosecond transients implies that a) only a few low-frequency vibrational modes are strongly activated upon impulsive population of the excited state and b) these vibrational modes are underdamped. The latter result may seem surprising in view of the manifold of vibrational modes of the protein with which energy might be exchanged. The specific structure of the protein 'solvent' and the reduced degrees of freedom of the surrounding membrane (two-dimensional) solvent may prevent the fast randomization of the activated motion.

The ability to directly vizualize vibrational motion in a protein enables studies of the relation between nuclear motion and functioning. In the case of reaction

centers the coherence time of the observed vibrations strongly suggests that the highly efficient primary charge transfer reaction does not take place in a regime of thermally equilibrated vibrational motion, as usually assumed in conventional theories (Bixon and Jortner, 1986; Marcus and Sutin, 1985). The oscillatory features have mainly been studied at cryogenic temperatures, but they are also observable, albeit much weaker, at physiological temperatures (Vos et al., 1993). In the present paper the temperature dependence of the oscillatory features is analyzed in terms of the construction of the initially created wave packet and of the dephasing mechanisms.

The protein under study is a genetically modified bacterial reaction center from the purple bacterium *Rhodobacter capsulatus*, the D_{LL} mutant, which has been constructed by D.C. Youvan and his collaborators at MIT (Robles et al., 1990a, b). This mutant lacks the bacteriopheophytin acceptor, so upon excitation directly into the lowest electronic transition (P_-) the system evolves on the P^* excited state potential energy surfaces only on the picosecond time scale. Detection in the stimulated emission region, at lower energy than the P_- transition, ensures, in principle, that only excited state dynamics, and no ground state dynamics, are monitored. Oscillatory features which have the fundamental frequencies of the vibrational motions they reflect, are best observed at both sides of the stimulated emission spectrum, i.e. when probing the turning points of the potential energy surface. The detection wavelength in this study is 945 nm, which is at the red side of the stimulated emission spectrum for all temperatures.

Materials and Methods

Chromatophores of *R. capsulatus* D_{LL} devoid of antenna proteins were prepared at MIT by S.J. Robles and D.C. Youvan as described elsewhere (Robles et al., 1990a, b). The samples were diluted in glycerol (60% vol/vol) to an optical density of 0.5 at 870 nm (at room temperature) in a plexiglass cuvette with an optical path length of 1 mm. The samples were cooled in the dark in a convection cryostat with helium (< 100 K) or nitrogen gas. The temperature was measured with a calibrated thermodiode and maintained within ± 3 K. At each temperature, the sample was equilibrated for at least 15 minutes before each measurement. At intermediate temperatures, results obtained after cooling of the sample to a given temperature were identical to results obtained after warming to the same temperature.

The experimental apparatus is described in detail elsewhere (Martin and Vos, 1993). Briefly, pump pulses centered at 870 nm (spectral width 10 nm and temporal width 80 fs) were used to excite $\sim 20\%$ of the reaction centers with a repetition rate of 30 Hz. Continuum probe pulses, compressed to 30 fs at the detection wavelength, were used to monitor the excited state dynamics at $\lambda = 945$ nm, in the stimulated emission region of the excited state P^*. The relative transmission was obtained by shot-to-shot normalizing the intensity of the probe beam

to the intensity of the reference beam which does not pass through the sample. Noise due to fluctuations in the pump beam was minimized by normalizing the transmission change to the pump beam intensity at each shot.

Results

As charge separation does not take place in the D_{LL} mutant due to the absence of the bacteriopheophytin acceptor H_L, the excited state remains populated for a few hundred picoseconds (Breton et al., 1990). The overall stimulated emission therefore does not decay within a few picoseconds, but oscillations are superimposed on the kinetics, which has been interpreted as reflecting vibrational motion in the excited state (Vos et al., 1993). Fig. 1 shows that the oscillations are

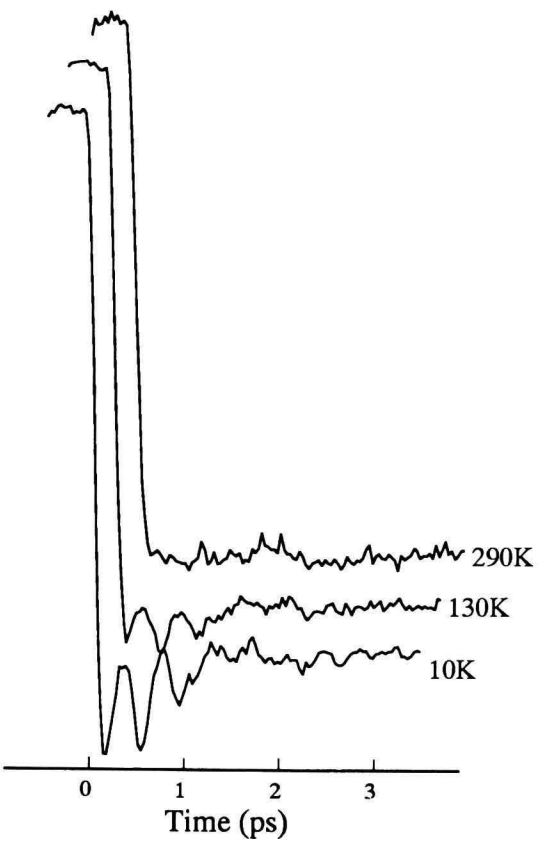

Fig. 1. Kinetics of stimulated emission at selected temperatures of chromatophores of *R. capsulatus* containing D_{LL} mutant reaction centers and devoid of light harvesting proteins. 80 fs Fourier transform limited pump pulses centered at 870 nm and 30 fs continuum probe pulses were used. The detection wavelength was 945 nm. The traces are normalized to the average amplitude of the overall signal.

most prominent at 10 K (amplitude $\sim 20\%$ of total signal) but are still clearly present at room temperature (amplitude $\sim 4\%$ of total signal). The oscillatory parts, isolated by subtracting a heaviside function from the data, (Fig. 2) show that increasing the temperature mainly causes a decrease in the amplitude of the oscillations, and does not strongly affect the damping of the oscillations.

The data were further analysed by Fourier transforming the data after the pump-probe overlap time (Fig. 3). Two bands (low temperature peaks at 15 and 77 cm^{-1}) are present. The 77 cm^{-1} band clearly broadens at higher temperatures and disappears at room temperature. The 15 cm^{-1} band decreases somewhat slower and broadens clearly only at 290 K. The limited spectral resolution (± 8 cm^{-1}) may mask broadening effects at higher temperatures. Fig. 4 shows the normalized amplitude of the two peaks at 15 and 77 cm^{-1} as a function of temperature.

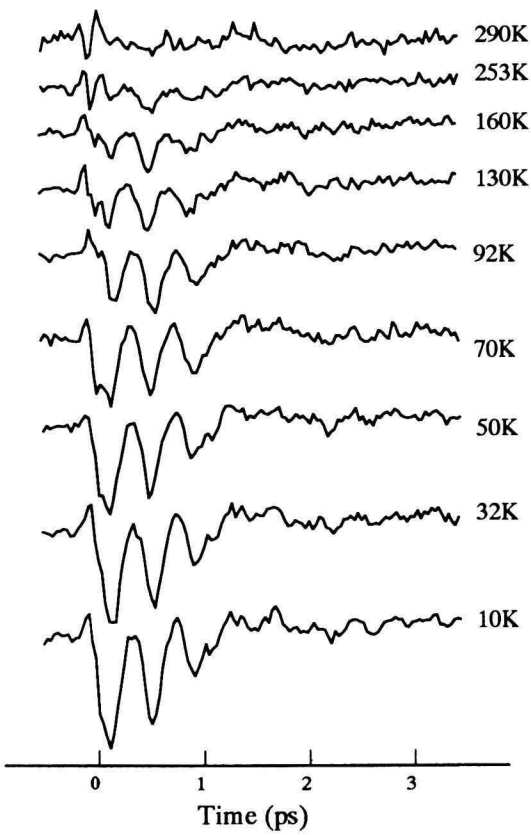

Fig. 2. Oscillatory parts of the data of Fig. 1 and of data obtained at intermediate temperatures. The oscillatory part is obtained by taking the residuals of a fit to a step function convoluted with the instrument response function.

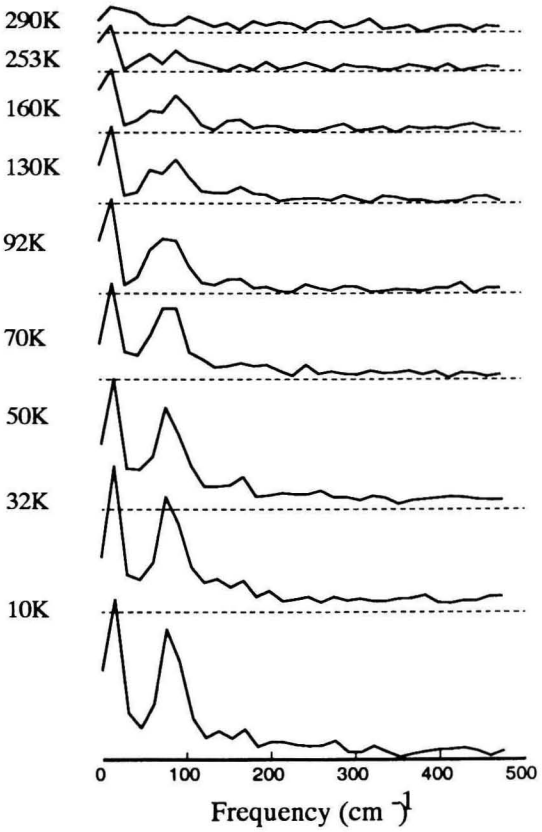

Fig. 3. Fourier transforms (FT) of the oscillatory parts of Fig. 2. Discrete FT describes the input signal $I(t)$ by its amplitudes A and phases ϕ as a function of the frequency ν as $\Sigma_n A_n Re(e^{i(2\pi\nu_n t - \phi_n)})$ (Re: real part) and was taken over a 2.13 ps data interval (64 points), yielding a spectral resolution of 15 cm^{-1}. The FT window was started at $t = 0.10$ ps, i.e. excluding the pump-probe overlap time interval. The spectral region above $\sim 200\ cm^{-1}$ represents the noise level of the data.

Discussion

We will discuss our results in terms of wave packet preparation and its subsequent time evolution on the excited state potential energy surface. The temperature is expected to affect both the efficienty of the dephasing process of the vibrational wave packet through collisional type of events and the quality of the impulsive preparation of the initial wave packet from the ground state, which vibrational levels are thermally populated in the steady state.

The damping observed in the femtosecond kinetics may be due to dephasing of the wave packets through collisions which randomizes the nuclear motion (incoherent dephasing). On the other hand, damping may also be due to frequency dispersion (coherent dephasing). In the latter case, a distribution of vibrational modes or levels is activated with slightly differing frequencies. The

initial phase correlation is then lost in a time related to the width of the frequency distribution, but the phase memory within each vibrational level is not lost and the distribution may in principal rephase (cf. Dantus et al., 1990). At 10 K the observed oscillations damp in about 2 ps for both the 15 and the 77 cm^{-1} mode (Figs. 1 and 2). If incoherent dephasing processes were the main source of the damping of the oscillations at 10 K, one would expect a decrease of the damping time upon warming, as the low frequency modes are expected to couple more strongly to the bath at higher temperatures. The fact that this is not observed (Fig. 2) suggests that incoherent dephasing is at least not the only source of dephasing; hence the incoherent vibrational dephasing time probably exceeds the lower limit of ~ 1 ps.

The temperature dependence of the initial preparation of the wave packet thus may be largely responsible for the decay of the amplitude of the oscillations at higher temperatures. The population distribution of the excited state vibrational levels is mainly determined by the pump bandwidth (130 cm^{-1}) at 10 K ($kT=$ 8 cm^{-1}) and by the ground state Bolzmann distribution at 290 K ($kT = 235$ cm^{-1}). Increasing the temperature from 10 K to 290 K relatively increases the initially populated excited state levels in a very similar manner: from ~ 9 to ~ 16 levels for the 15 cm^{-1} mode and from ~ 2 to ~ 3 for the 77 cm^{-1} mode. This may explain the general similarity of the amplitude decreases for both features (Fig. 4).

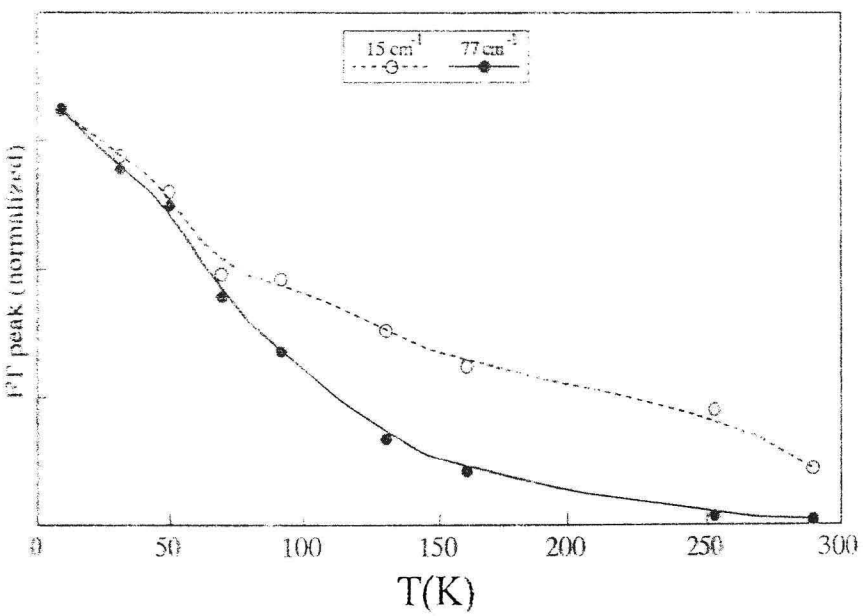

Fig. 4. Dependance of the amplitude of the FT peaks at 15 and at 77 cm^{-1} of Fig. 3 on the temperature. The curves are normalized at 10 K.

Apart from the distribution of vibrational levels, also the distribution of the initial phases within the wave packet may be affected by the temperature. At low temperature the ground state velocities are small and for a displaced mode the initially prepared wave packet will be near the turning point (where the wave packet velocity is zero). At higher temperature the initial velocities are higher and the phase of the wave packet is less well defined, leading to broader wave packets and lower amplitudes of the oscillations. Finally, the site inhomogeneous broadening ($\sim 170\,cm^{-1}$ at 10 K (Johnson et al., 1990)) probably increases at higher temperature, although no data are available on this issue. This would lead to effective broadening on warming of the distribution of levels excited by the pump pulse and of the distribution of nuclear coordinates probed by the probe pulse.

The decay of the amplitudes of both oscillatory features (Fig. 4) is roughly similar, but the decay of the amplitude of the $15\,cm^{-1}$ feature is less pronounced. This effect may in part be due to the fact that the relative broadening of the two features more strongly affects the peak amplitude of the higher frequency component, as the Fourier transform is discrete. Further, it seems that the broadening is biased towards higher frequencies, which selectively reduces the $77\,cm^{-1}$ component, which is at the edge of the vibrational bandwidth for the experiments (due to the 80 fs pump pulse).

A more quantitative analysis of the temperature dependence of the amplitudes of the oscillatory features must await the measurement of the probe wavelength dependence at different temperatures, as the maxima and width of the absorption, and presumably the stimulated emission, spectra are temperature-dependent. Nevertheless, an important result from this work for the functioning of reaction centers is that phase conservation also persists at physiological temperatures, thus stressing the possible importance of vibrational coherence for the primary electron transfer reaction in wild type reaction centers. In this latter case we will have to reconcile our observations with those from other groups using different experimental approaches including hole burning and resonance Raman experiments.

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