Electric Field Effects on IR Absorption of α-Helical Polypeptide

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External electric field effects on the IR absorption spectrum of α-helical poly(γ-benzyl-L-glutamate) have been examined with electric field modulation spectroscopy. The electric-field-induced change in each vibrational band is reproduced by a superposition of the absorption spectrum, and its first and second derivative spectra, suggesting that the electric dipole moment and the molecular polarizability change following vibrational excitation. The N–H stretching band at 3291 cm^{-1} shows the largest change in the magnitude of dipole moment following vibrational excitation.

Vibrational spectra of synthetic polypeptides have been examined extensively to understand the structural and dynamical properties of proteins.[1–4] While synthetic polypeptides lack the complexity of naturally occurring proteins, they exhibit the conformations that are similar to the protein secondary structures. The frequencies and intensities of polypeptide vibrational bands reflect the orientation and position of polypeptide backbones and side chains.[1–4] Poly(γ-benzyl-L-glutamate) (PBLG) is a well-known polypeptide that exhibits several conformations, depending on the molecular weight and preparation. Many researchers have reported the vibrational spectra of PBLG to establish the correlation between the spectral pattern and the secondary structure.[5]

Measurements of electroabsorption spectra (plots of the electric-field-induced change in absorption intensity as a function of wavenumber) provide unique information on electronic structures of molecules or molecular systems.[6,7] In fact, we have determined the changes in electric dipole moment ($\boldsymbol{\mu}$) and molecular polarizability ($\boldsymbol{\alpha}$) following photoexcitation to electronically excited states using a field modulation technique in absorption spectroscopy.[6,8] It is also expected that a wealth of information on molecular structures is obtained from the analysis of field-induced changes in vibrational spectra (vibrational Stark effect). Electroabsorption spectra in the IR region have been recently measured by several groups;[9–11] to our knowledge, however, no one has reported electric field effects on vibrational spectra of polypeptide backbones.

In the present study, we have measured the electroabsorption spectrum of PBLG with an α-helix conformation in the IR region. We have analyzed the electric field effects on IR absorption bands of the polypeptide and evaluated the change in $\boldsymbol{\mu}$ following each vibrational excitation. Hereafter, electroabsorption spectrum in the IR region is abbreviated as IR E–A spectrum and applied electric field is denoted by $\boldsymbol{F}$. The chemical structure of PBLG is depicted in Figure 1a.

PBLG (MW = 150,000–350,000) was purchased from Sigma Chemical Co. Unoriented solid films of PBLG were cast from dioxane solution on a semitransparent aluminum (Al) coated $\mathrm{CaF_2}$ substrate. Then, another semitransparent Al film was deposited on the dried polymer film by a vacuum vapor deposition technique. The two Al films were used as electrodes. The thickness of the polymer film was evaluated by FIB-SEM measurements. IR E–A spectra were recorded on a DIGILAB FTS7000e FT-IR spectrometer. Applied voltage was a repetition of rectangular waves of positive and zero bias in turn. Alternative interferogram scans with and without $\boldsymbol{F}$ were repeated many times, and the common logarithm of the ratio of the averaged spectrum without $\boldsymbol{F}$ to that with $\boldsymbol{F}$ gives an IR E–A spectrum.[9,10] All the measurements were carried out at room temperature in air.

Figure 1 shows the IR absorption spectrum and the IR E–A spectrum of the PBLG polymer film prepared in the present study. The vibrational assignments of PBLG have been well established;[1,5] the assignments of some prominent bands are shown in this figure. From the wavenumbers of the observed amide I (1653 cm^{-1}) and II (1549 cm^{-1}) bands, it is confirmed that PBLG exists in the α-helix conformation in the prepared film.[1,5] The shape of the IR E–A spectrum is remarkably different from that of the original IR absorption spectrum; each vibrational band shows characteristic field dependence.

According to the theory of electric field effects on optical spectra derived by Liptay and co-workers,[12] the field-induced change in absorption intensity of an isotropic sample at wavenumber ν, i.e., $\Delta A(\nu)$, is given by a sum of the zeroth, first, and second derivatives of the absorption $A(\nu)$ as follows:

$$\Delta A(\nu) = (f\boldsymbol{F})^2[\mathrm{A}'A(\nu) + \mathrm{B}'\nu\{\mathrm{d}(A(\nu)/\nu)/\mathrm{d}\nu\} + \mathrm{C}'\nu\{\mathrm{d}^2(A(\nu)/\nu)/\mathrm{d}\nu^2\}], \tag{1}$$

where f is the internal field factor. The coefficient A′ depends on the transition polarizability and the molecular orientation. B′ is mainly attributed to the difference in molecular polarizability

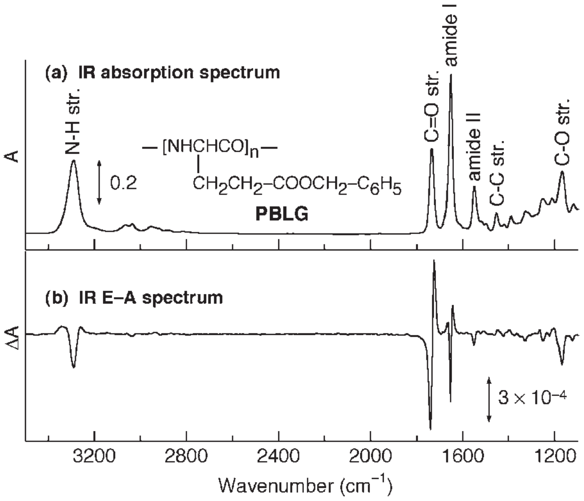

Figure 1. (a) IR absorption spectrum and (b) IR E–A spectrum of PBLG. Applied field strength was 0.6 $\mathrm{MV\,cm^{-1}}$.

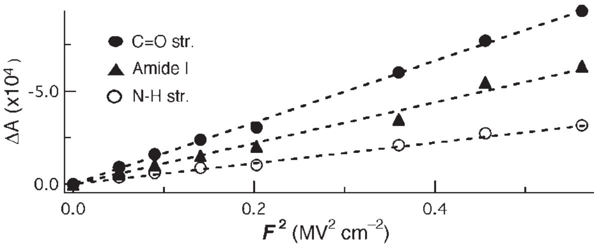

Figure 2. Plots of the field-induced change in IR absorption intensity of PBLG as a function of the square of the applied field strength.

between the ground (g) and excited (e) states, i.e., $\Delta\alpha = \boldsymbol{\alpha}_e - \boldsymbol{\alpha}_g$. C′ depends on the difference in electric dipole moment between the ground and excited states, i.e., $\Delta\boldsymbol{\mu} = \boldsymbol{\mu}_e - \boldsymbol{\mu}_g$, which is given by the following equation:

$$C' = |\Delta\boldsymbol{\mu}|^2[5 + (3\cos^2\xi - 1)(3\cos^2\chi - 1)]/(30h^2c^2), \quad (2)$$

where χ is the angle between the direction of $\boldsymbol{F}$ and the electric vector of the excitation light, and ξ is the angle between the direction of $\Delta\boldsymbol{\mu}$ and the transition dipole moment.

The magnitudes of ΔA are shown to be proportional to the square of the applied field strength (see Figure 2). The shape of the IR E–A spectrum remains unchanged over the range of fields we have applied. The E–A spectrum in every IR band is simulated by Eq 1, which is shown in Figure 3. All the bands are satisfactorily reproduced by a linear combination of the absorption spectrum and its first and second derivative spectra. These results indicate that the theory of Eq 1 can also be applied to the IR E–A spectrum of a polypeptide.

As shown in Figure 3, with respect to main-chain vibrations, both the N–H stretching (3291 cm^{-1}) and amide I bands give a shape similar to the second derivative of the absorption spectrum. This indicates that the magnitudes of ΔA of these two bands essentially come from $\Delta\boldsymbol{\mu}$ following vibrational excitation. On the other hand, the zeroth and first derivative components significantly contribute to the IR E–A spectrum of the amide II band. With respect to the side-chain vibrations, the C=O stretching band of the ester at 1734 cm^{-1} exhibits the largest magnitude of the field-induced change. The IR E–A spectrum of the C=O stretching band is similar in shape to the first derivative of the absorption spectrum. The phenyl C–C stretching band at 1454 cm^{-1} also shows the profile of the first derivative, while the zeroth component is dominant for the ester C–O stretching band at 1169 cm^{-1}.

The magnitude of $\Delta\boldsymbol{\mu}$ following vibrational excitation can be obtained using Eq 2. Under the present experimental condition of $\chi = 90°$, the $|\Delta\boldsymbol{\mu}|$ values of the N–H stretching, amide I, and ester C=O stretching bands are evaluated to be 0.11, 0.05, and 0.06 D/f, respectively, with $\xi = 0°$.[2,5,9] The magnitudes of $\Delta\boldsymbol{\mu}$ of other IR bands are found to be in the range of 0–0.03 D/f, indicating that the $|\Delta\boldsymbol{\mu}|$ value of the N–H band is much larger than the others. Owing to the largest magnitude of the first derivative component, the ester C=O stretching band may be considered to have a marked $|\Delta\boldsymbol{\alpha}|$ value. The qualitative value of $|\Delta\boldsymbol{\alpha}|$ is 2.0 Å^3/f^2;[8,9] however, measurements of the χ-dependence of the IR E–A spectrum are necessary to perform the quantitative evaluation of $\Delta\boldsymbol{\alpha}$.

The zeroth negative component arises from a slight orientation of PBLG along $\boldsymbol{F}$,[13] since the transition polarizability causes the positive value of A′ for one-dimensional systems such as a C=O or N–H stretching mode.[7] The magnitude of the zeroth component is larger in the side-chain vibrational region than in the main-chain vibrational region, suggesting that the side chain is oriented more significantly in the presence of $\boldsymbol{F}$. Such an orientation induces dielectric polarizability; information on dielectric constants of polymers at a molecular level will be obtained by the analysis of the zeroth components of IR E–A spectra.

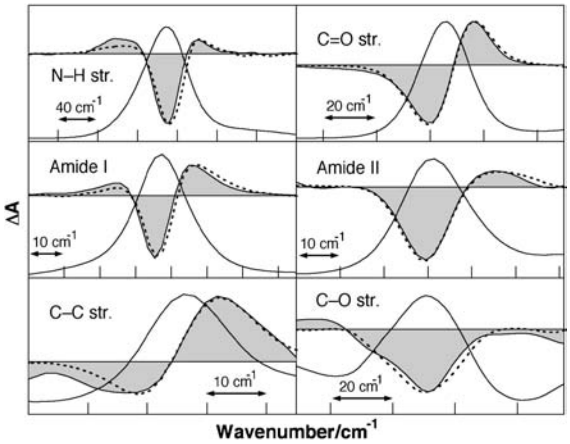

Figure 3. IR E–A spectrum (shaded line) and IR absorption spectrum (solid line) of PBLG around six prominent band regions. The simulated IR E–A spectrum is also shown by a dotted line in every case.

In conclusion, each vibrational band of polypeptide exhibits characteristic dependence on $\boldsymbol{F}$ and the magnitude of $\Delta\boldsymbol{\mu}$ following vibrational excitation is evaluated using Eq 2. We believe that measurements of IR E–A spectra will give new scope for elucidating the structures of polypeptide molecules.

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