Temperature-dependent Photodegradation in UV-resonance Raman Spectroscopy

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Temperature-dependent photodegradation during UV-resonance Raman spectroscopy was investigated. Photodegradation was quantitatively probed by monitoring the temporal evolution of UV-resonance Raman spectra obtained from bacteriochlorophyll (BChl) showing, resonance effect at a 355-nm excitation wavelength. At 80 K, the molecular photodecomposition rate was 5-times lower than that at room temperature. The decomposition rates of BChl were analyzed by the Arrhenius formula, indicating that the mechanism of photodegradation includes a thermal process having an activation energy of 1.4 kJ/mol.

Keywords UV-resonance Raman spectroscopy, photodegradation, Arrhenius formula, photothermal effect

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The Raman peaks of the sample analyzed at 80 K were of higher intensity than those of the sample analyzed at 300 K. Many weak BChl Raman peaks that were difficult to distinguish in the spectrum at 300 K were clearly visible in the 80 K spectrum, indicating that conducting UV-resonance Raman spectroscopy at lower temperatures effectively prevents sample photodegradation. In addition, the line width at 80 K was sharper, indicating better spectral separation.

The dominant Raman peaks at 1611, 1530, 1343, and 1287 cm$^{-1}$ originating from the conjugated macrocycle of BChl were used for further time-dependent analyses, because these peaks were well isolated, firmly assigned, and showed a reasonable S/N ratio after a long exposure. The 1611 cm$^{-1}$ peak, which had the highest frequency, can be attributed to $C_a$-$C_a'$ stretching, whereas the peak at 1530 cm$^{-1}$ represents coupled vibrations of $C_a$-$C_b$ and $C_a$-$C_c$ stretching, and the peaks at 1343 and 1287 cm$^{-1}$ represent coupled vibrations of $C_r$-$C_m$ and $C_r$-$N$ stretching and CH in-plane bending. An additional peak at 1065 cm$^{-1}$ was assigned to stretching of the C-C bonds outside the conjugated system but within the macrocycle. The peak observed at 1568 cm$^{-1}$ on the left shoulder of the 1611 cm$^{-1}$ peak only appears in the 300 K spectrum, and can be assigned as a photoproduct because it appeared after a long exposure.

For the quantitative analysis of photodegradation at different temperatures, the temporal evolution of the Raman bands was characterized. Figure 2 shows the time-dependent Raman intensity of BChl at 4 different temperatures of 300, 200, 150, and 80 K. The data were normalized by the intensity of the initial data point (0 min). The intensity was extracted from the spectral heights determined by least-square fittings of the Lorentzian function. The decay curves were generated by averaging the time dependence of 4 peaks attributable to intact BChl. Our experimental conditions with the CW UV excitation laser used in this experiment would not cause bond-selective decompositions. Therefore, we assumed that the intensity decay rates of the 4 peaks were all equivalent to the decomposition rate of the molecule. Figure 2 clearly illustrates that the BChl decomposition rate decreases with decreasing temperature. The fluctuations become larger over time because the signal intensity become weaker due to the accumulated irradiation. After 30 min, no further spectral evolution was observed.

The decomposition rate was calculated by curve fitting using

$$I = I_0 \exp \left(-\frac{t}{\tau}\right) + y_0, \quad (1)$$

where $I$ represents the experimentally determined signal intensity at time $t$, $\tau$ represents the decay time constant, and $I_0$ and $y_0$ represent the initial and background intensity which is in common with all spectra. The value $\tau$ for each temperature examined are shown in the inset of Fig. 2. These data show that the time constant increases as the temperature decreases. The decay constant at 80 K was approximately 5-times higher than at room temperature, indicating that 5-times more signal can be detected.
accumulated at 80 K in UV-resonance Raman spectroscopy, leading to better S/N ratios in analyses of soft materials. The typical bond dissociation energies of organic compounds are around 300 - 600 kJ/mol, equivalent to the photon energy of light at wavelengths in the range 396 - 198 nm. At excitation wavelengths exceeding the bond-breaking energies, thermal activation energy is the only contributor to sample degradation. On the other hand, at shorter excitation wavelengths, direct ionization of molecules become obvious. From this point of view, an excitation wavelength of 355 nm could produce the optical dissociation of chemical bonds. Resonant excitation of the Soret band may lead to the formation of radical cations or oxidized states. However, if the short wavelength is the only contribution to the sample degradation, such a temperature effect should not be observed.

To understand the origin of the molecular photodegradation process, further consideration of the experimentally obtained $\tau$ is helpful. Figure 3 shows the relationship between the rate constant, $k = 1/\tau$, and the temperature, $T$. The experimental results revealed that a linear relationship exists between $1/T$ and $\ln(k)$, which would be expected from the Arrhenius formula:

$$\ln(k) = C_0 + C_1 \cdot 1/T$$

(2)

where $C_0$ represents the natural log of the frequency factor and $C_1$ represents the activation energy over the Boltzmann constant. The activation energy obtained from the Arrhenius plot in Fig. 3 was 1.4 kJ/mol, which is much smaller than the direct bond-breaking energy of typical organic molecules. Under UV irradiation, a molecule is easily pumped to an electronically excited state. The reaction pathway of the excited-state-reactant and the product can be smaller compared to the one from the ground-state-reactant. We guess that the low-temperature environments prevent the molecule from overcoming the small potential barrier. The energy of $\sim 1$ kJ/mol is equivalent to the activation energy of inner-molecular bond rotation. In the case of a macromolecule, such as BChl, the inner-molecular rotation plays an important role for chemical reactions, which will lead to degradation of the molecule.

For the first approximation, we analyzed the decay constant as a single exponential function in order to obtain a better understanding of the effect of temperature. However, photodegradation mechanisms based on direct photo-ionization or radical formation could also be involved in the decay process. The energy dissipation pathways in a molecular system, which are likely to be very complex, should be taken into account when evaluating molecular photodegradation at different temperatures.

Finally, we would like to point out that low temperatures are always realized under a vacuum. Oxygen plays a significant role in radical formation and the quenching of excited states. The quenching of molecular excited states can lead to the suppression of molecular photodegradation, whereas the generation of radicals can have the opposite effect. Our observations indicate that the sample decomposition rate under a vacuum is approximately 2.4-times lower than that at the ambient atmosphere and 300 K. This suggests that oxygen may not suppress photodegradation via quenching, but rather may serve as a source of destructive radicals. Compared with ambient conditions, low-temperature measurements enable an even more effective avoidance of both thermal- and oxygen-associated disturbances.

**Conclusions**

In this study, we examined the temperature effect on photodegradation in UV-resonance Raman spectroscopy through the signal from BChl, having a resonance Raman effect at the incident laser wavelength (355 nm). The mechanism of the photodegradation process was discussed in relation to the signal decay constants, which were shown to follow the Arrhenius formula. It can be concluded that the observed photodegradation in UV resonant Raman spectroscopy involves the thermal process with an activation energy of 1.4 kJ/mol. Also the low temperature environment is indeed effective for less damage to biomolecular imaging.

**Supporting Information**

Fig. S1: Schematic illustration of the temperature-controlled UV-resonance Raman spectroscopy system. Fig. S2: UV-vis absorption spectra of BChl. Fig. S3: Raman spectra of BChl obtained by 532 nm laser excitation. The spectra was measured by Raman11 system (NanoPhoton Co.) under atmospheric pressure using an objective lens (magnification $\times 20$, 0.45 NA) with the same laser power and accumulation time as used in 355 nm excitation. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

**References**