Molecular Functionalization of Cold-Plasma-Treated *Bombyx mori* Silk

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Summary: *Bombyx mori silk* treated by cold SF_6 plasma was found to show higher hydrophobic property than the original silk. In order to clarify the chemical changes occurring in this treatment, the changes of functional groups of silk surface were investigated by attenuated total reflection (ATR) infrared spectroscopy, surface charge determination, and quantum mechanical calculation. Infrared spectra of original and plasma-treated silks do not show any change in the frequency regions of amide I, II, and III bands which locate at around 1627, 1513, and 1228 cm^{-1}, respectively. Slight changes were detected for the peak intensities of the bands locating in the frequency region of 1000–1050 cm^{-1} after plasma treatment. This suggests the formation of CF groups in the *Bombyx mori silk* chain. The zeta potential experiment suggested that the electrostatic charges of the silk surface were not affected by the plasma treatment. In order to investigate the surface state of the plasma treated silk, the density functional calculation was performed for the model compounds with similar chemical structure as that of *Bombyx mori silk*. In this calculation, a fluorine radical was located at the various positions of the model compound, and the energetically most plausible structures were extracted to show the chemical reaction of $CH + F^- \rightarrow CF + H^-$.

Keywords: *Bombyx mori silk*; cold SF_6 plasma; computer simulation

Introduction

At present stage, the use of plasma to modify surface properties of materials is experiencing rapid growth. The advantage of this technique is that plasma treatment changes only the uppermost atomic layers of a material surface without interfering the bulk properties. For example, *Bombyx mori* silk has been treated in a low-temperature SF_6 radio frequency of around 50 W with a pressure of 3–5 mTorr and found to increase the hydrophobic property of silk surface. After plasma treatment, the presence of CF–CF and $–CH_2–CHF–$ groups were found. High-resolution XPS spectra indicated the present of chemical bonding of fluorine on the treated surface.[1–4]

In this study, the change in functional groups of fibers surface were analyzed by attenuated total reflectance/infrared spectroscopy (ATR/IR) and Raman spectroscopy whereas electrical properties of the surfaces were characterized on the basis of zeta potential measurement. The wide angle X-ray diffraction (WAXD) and small angle X-ray scattering (SAXS) were used to determine the aggregation structure of these polymers. In molecular level investigation, the molecular quantum simulation works have been performed. As a method of calculation, we have chosen the BLYP functional of the generalized gradient

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approximation (GGA). Physical, chemical, and hydrophobic properties were investigated in order to clarify the functionalization of *Bombyx mori silk* surface.

Experimental Part

Materials

Bombyx mori silk fabric (GRAZIE, 52.9 g/m2, Thanapaisal R.O.P, Thailand) was treated utilizing an inductively coupled 13.56 MHz rf plasma system described elsewhere.[3] The 60×60 cm cylindrical plasma chamber is powered about 50–100 W. The sample (7×7 cm) was suspended in the middle of plasma chamber, 6–21 cm apart from the antenna. Working pressure of SF_6 gas was retained at 2.5 Pa. The treatment time was normally around 10 minutes.

X-ray Scattering Measurement

A Rigaku Nanoviewer (Micro-source generator, MicroMax 007, Japan) equipped with a rotating Cu anode generator ($\lambda = 1.5418$ Å) and coupled with a Confocal Maxflux Mirror was used at 40 kV and 20 mA. The scattering pattern was measured using an imaging plate and the 2D image was read by TRY XIA-23×25 IP reader. The exposure time was 4 hr for WAXD and 12 hr for SAXS.

Raman and IR Spectroscopic Measurements

The infrared spectra were measured with a Varian FTS 7000 series FT-IR spectrometer with 64 scans at a resolution power 4 cm^{-1}. The reflective spectra were collected using diamond crystal plates of MIRacle ATR accessory. Raman spectra were collected using a Jasco NRS-2100 green-laser Raman spectrophotometer at a resolution power 4 cm^{-1}.

Surface Charge Determination

The untreated and treated fibers were cut to the size of about 1 mm. The 0.10% w/v solids were prepared in 0.0010 M KCl at pH range 2–11 and stirred using magnetic stirrer for 10 minutes. The zeta potentials were then measured using zeta meter (Zeta meter 3.0).

Computer Simulation

The reaction mechanism of silk treated by plasma ion was tried to clarify using DMol3 module of Material Studio program. The nonlocal exchange and correlation energies of the reactant, transition states, and products of silk treated by plasma were calculated with the BLYP functional of the generalized gradient approximation (GGA). A Fermi smearing of 0.005 hartree was used to improve computational performance. The geometries of all stationary points were fully optimized. Frequency analysis at the same level determines the nature of the stationary points and each transition state with one imaginary frequency. The linear synchronous transit (LST) and quadratic synchronous transit (QST) methods were used to study the transition state.

Results and Discussion

Characterization of Plasma-Treated Bombyx mori Silk

A comparison between the ATR/IR spectrum of original silk (A) and silk treated by SF_6 plasma (B) is made as shown in Figure 1a. We observe common absorption bands of amide I (C=O stretching) at 1627 cm^{-1}, amide II (N–H deformation) at 1513 cm^{-1}, and amide III (C–N stretching) at 1228 cm^{-1}. There are changes of peak intensity located at 1000–1050 cm^{-1} within spectra A, which could be explained by the appearance of the bands characteristic of the stretching vibration of C-F bond.[5] The peak intensity changes are relatively low for both spectra due to the low concentration of fluorine on the treated SF surface. The slight change of the peak position can also be observed from Raman measurement in Figure 1b. A comparison between the ATR/IR spectra of silk treated by SF_6 plasma at difference sample-to-antenna distance of 6, 11, 16, and 21 cm was made as shown in Figure 2. There is an increase in the peak intensity corresponding to the C-F

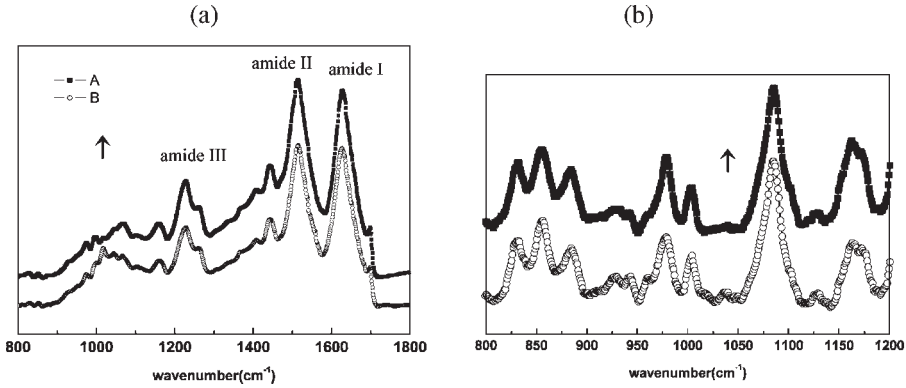


Figure 1.
(a) ATR/IR and (b) Raman spectra of *Bombyx mori silk* treated by SF_6 plasma.

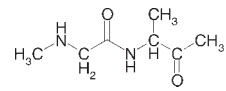
bond stretching when the sample-to-antenna distance $\leq$11 cm.

An electrokinetic property of *Bombyx mori silk* was studied via zeta potential measurement. The zeta potentials were investigated over the pH ranges of 2–11. For untreated silk, it exhibits a negative charge ($\zeta_{\text{platau}} = -40$ mV) on the surface in the range of pH 4–11. The similar trend was also shown for the treated sample. In addition, points of zero charges for untreated and treated samples are not significantly changed. It indicates that the pre-plasma-treated silk fiber slightly affects on the presence of negative charge on the surface. This appearance may occur as a result of the effect of plasma concentration used for pretreatment process.

The WAXS patterns of all silk samples indicate the present of crystalline structure in high portion. The SAXS patterns show a strong central diffuse scattering. This pattern indicates that voids dominate the central scattering so called the void streak found in silk fibers from *Bombyx mori*. No change in diffraction pattern among various sample-to-antenna distance plasma treated silk was found. So the plasma treatment at low temperature does not affect the bulk region.

Computer Simulation

The crystal structure of *Bombyx mori silk* can be simplified as a repetition of alanine (Ala) and glycine (Gly) linked with β-pleated conformation.[6–8]



Model compound of *Bombyx mori silk*

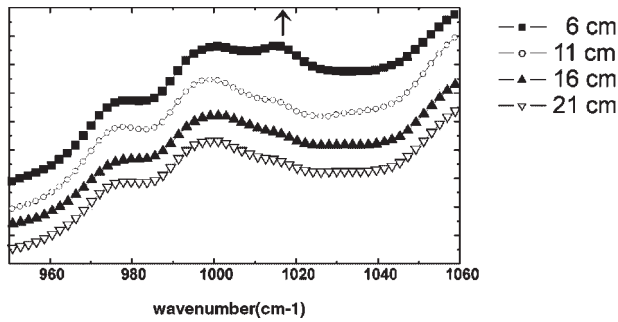


Figure 2.
ATR/IR spectra of *Bombyx mori silk* treated by SF_6 plasma at difference sample-to-antenna distance: A, and B spectrum are for the original and the silk treated by SF6 plasma, respectively.

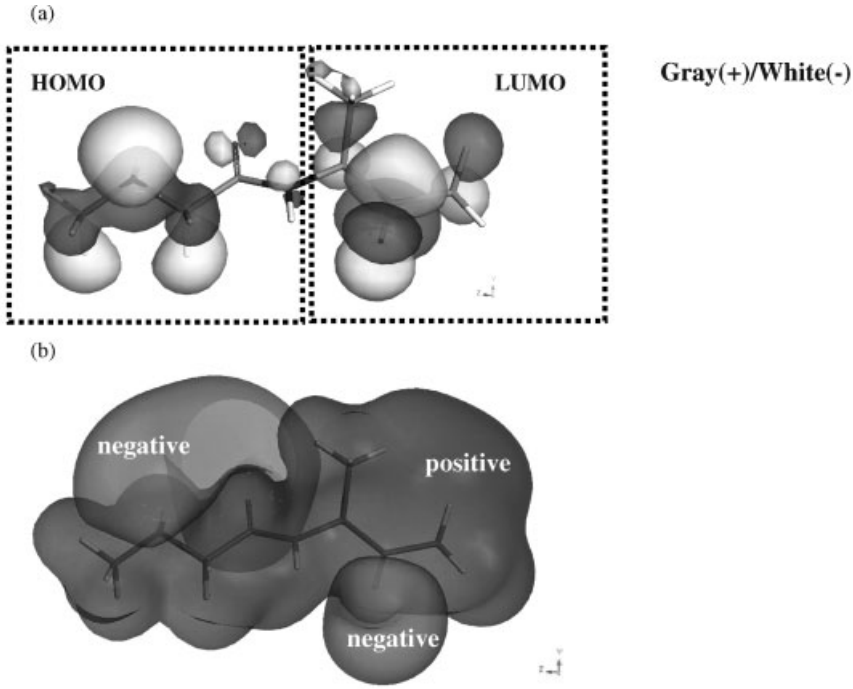


Figure 3.
(a) HOMO-LUMO and (b) electrostatic potential plots of *Bombyx mori silk* model compound.

Energy minimization was performed for the model compound of silk to obtain the optimized molecular conformation. Minimized molecular structure of the model compound is essentially the same as β-pleated conformation of protein, corresponding well to the crystallographic data. By considering the molecular orbitals of LUMO (Figure 3a) and the electrostatic potential energy profile (Figure 3b) of this model, an F anion in SF_6 plasma should react with methyl group of Ala part of silk model.

As shown in Table 1, we calculated three possible reaction pathways of F anion reacting with CH_4, C_2H_6, and NH_2–$CH(CH_3)$–CO–H to simulate side chain of Ala in order to search the reaction path possible for the silk macromolecule.

The energy barrier of each reaction shown in Table 1 is less than 3 eV which is in the range of experimental condition.[3] The reaction pathway of NH_2–$CH(CH_3)$–CO–H reacts with F anion was shown in Figure 4 for an example of the reactant, transition states, and products energies calculation.

Conclusions

In this work we have demonstrated a chemical characterization utilizing a combination of X-ray diffraction, ATR-IR spectroscopy, Raman spectroscopy, surface charge determination, and density functional calculation. The results indicate that the change in functional groups of *Bombyx mori silk* fibers modified by SF_6 plasma treatment may be detected as the creation

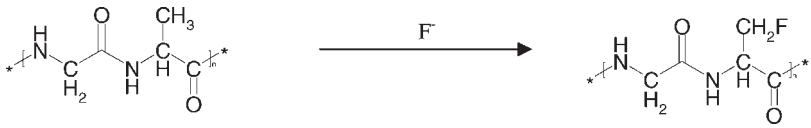
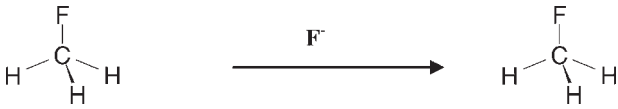
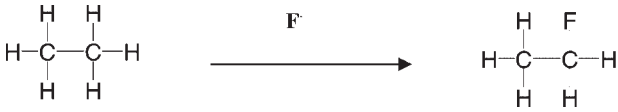
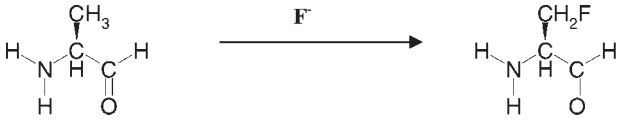


Table 1.
Energy barriers of F anion reacting with CH_4, C_2H_6, and NH_2–$CH(CH_3)$–CO–H reaction

Reaction	Energy barrier (eV)
	0.867
	0.958
	2.856
Experimental condition [3]	3–5

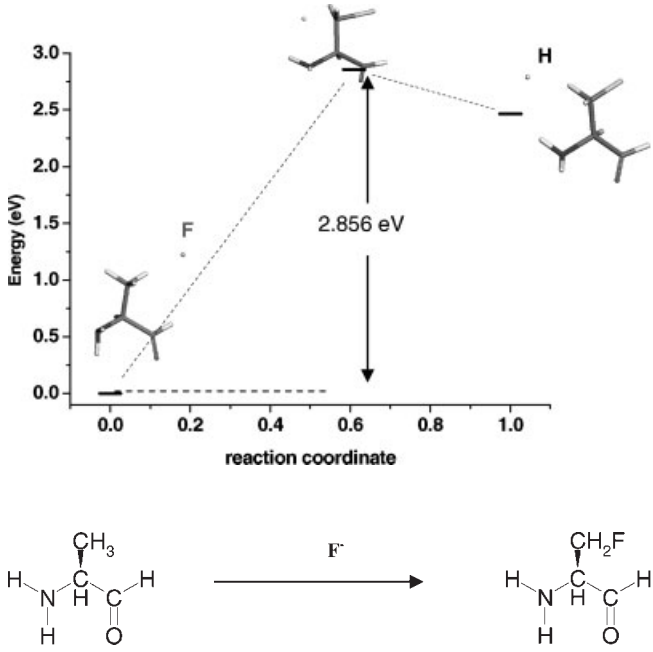


Figure 4.
Transition state calculations of NH_2–$CH(CH_3)$–CO–H reacting with F anion.

of CF group on the silk surface. This surface modification leads to the increase in hydrophobic property of silk surface after the SF_6 plasma treatment.

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