Sulfur hexafluoride plasma surface modification of Gly-Ala and Ala-Gly as *Bombyx mori* silk model compounds: Mechanism investigations

W. Sangprasert a, V.S. Lee a, D. Boonyawan b, K. Tashiro c, P. Nimmanpipug a,*

a Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
b Department of Physics, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
c Department of Future Industry-Oriented Basic Science and Materials, Graduate School of Engineering, Toyota Technological Institute, Tempaku, Nagoya 468-8511, Japan

**ABSTRACT**

Low-pressure plasma has been used to improve the hydrophobicity of Thai silk. In this study, Glycine–Alanine (GA) and Alanine–Glycine (AG) were chosen to represent model compounds of *Bombyx mori* silk. Single crystals of the simplified model compounds were characterized by polarizing microscopy and X-ray diffraction. The space groups of P2 1_2 1_2 1 and P2 1 were found for AG and GA, respectively. The initial structures for calculation were obtained from the experimental crystal structures. Density functional theory at the BHHandHLYP levels was used to investigate possible mechanisms of fluorine radicals reacting with AG and GA in the SF6 plasma treatment. The results indicate that hydrogen atoms of silk model compounds were most likely to be abstracted from the alanine residue.

**Keywords:**

Bombyx mori silk  
Plasma treatment  
DFT  
Transition state searching

**1. Introduction**

During the past several decades, of SF6 plasma has been employed to improve the hydrophobic properties of Thai silk [1–3]. Natural fibres are in general delicate and cannot be treated under extreme conditions. Plasma processing of textiles should thus be performed through low-pressure RF discharges of suitable gases. The resulting water absorption properties have been found to depend upon the treatment time, radio frequency (RF) power and gas pressures [2].

Thai silk, which is derived from the silk moth *Bombyx mori*, has a heavy chain that consists mainly of glycine (44%) and alanine (30%) [4–8]. The crystal structure of silk fibroin has been examined by several research groups using the constrained least-squares refinement [9]. The simplest model consistent with the X-ray scattering pattern is Gly-Ala or Ala-Gly [8]. Although these structures were solved earlier by Tranter et al. and Naganathan and Venkatesan [10–16], the Gly-Ala structure is polymorphic, indicating the flexibility and the potential of possible alternate structures.

Low-pressure plasma treatment is an environmental friendly process used for modification of wettability, shrink resistance, interfacial adhesion, hydrophilicity and dyeing properties of polymeric materials [17–22]. The main species of such plasmas have been delineated by Picard, who indicated three kinds of energetic species reacting on the polymer surface: neutral molecules, ions, and an electron density [23]. The studies of Joubert and Frank suggested that the reaction mechanism at the material surface in a plasma system was a two-step process for energetic species of fluorine reacting with the polymer surface [24,25].

In 2001, Selli et al. proposed a reaction mechanism for the silk surface treated with SF6 plasma. The reaction is initiated by very active species present in the plasma, such as ions, radicals, electrons and photons, most probably via a two-step replacement of hydrogen atoms. Using XPS, the ratios of F/C, O/C and N/C were determined [26]. The F/C ratio steadily increased with an increase of treatment time and pressure, while the O/C and N/C ratios exhibited no change following exposure to plasma. In 2001, Selli et al. reported hydrophobicity improvement of silk achieved by SF6 plasma treatment as reflected in the water repellence property measured by water contact angle [26,1,2]. In addition, a molecular model of *B. mori* silk and the fluorine atoms after the plasma treatment was investigated from the fundamental quantum mechanical level. Quantum mechanical calculations were applied to investigate the surface state of the plasma treated silk, using model compounds with similar chemical structure as that of *B. mori* silk.

The most plausible structures of fluorine radicals were extracted to examine the chemical reaction CH + F → CF + H [27]. To understand the mechanism of fluorine atoms in an SF6 plasma reacting with the silk surface, computational methods such as DFT have the potential to produce very accurate results at low cost [27–30].

One way to study complicated polymer structures is to use model compounds of polymeric crystalline structures. Tashiro et al. reported that both poly-para-phenyleneisophthalamide...
(PPTA) and poly-meta-phenyleneisophthalamide (PMIA) crystal structures can be well represented by computer simulations using the software Polymorph Predictor [31–34]. The present study thus aims to investigate the water repellent properties of B. mori silk, using model compounds and computer simulation. The model compounds Gly-Ala (GA) and Ala-Gly (AG) were employed in order to investigate the chemical modification of the silk surface resulting from the SF$_6$ plasma treatment process. Single crystals of the silk model compounds were prepared and their 3D structures elucidated. In parallel, from a theoretical point of view, QM calculations using the DFT method were used to investigate adsorption, interaction and possible mechanisms for the interaction of GA and AG with the fluorine radical (F$^-$).

2. Materials and methods

2.1. The plasma process

Powder of the simplified model compounds, NH$_2$CH$_2$CONHCH$_2$(CH$_3$)COOH and NH$_2$CH(CH$_3$)CONHCH$_2$COOH (GA and AG), were purchased from Sigma–Aldrich. The powdered forms were pressed into pellets with a hydraulic force of 10 and 7 N, respectively [28]. The pellets were exposed to SF$_6$ plasma, which was produced by an inductively coupled RF discharge at 13.56 MHz, as described elsewhere [3]. The RF generator, a Dressler model HPG1365, was connected to an antenna through a matching network. The diameter of the quartz chamber was 10 cm and the length was 16 cm. The operating gas, SF$_6$, was fed through a needle valve for operating pressure adjustment. The base pressure of the system was 1.6 mTorr.

The treatment conditions were optimized via adjustment of three parameters: pressure, RF power and treatment time. The optimum conditions were chosen in respect to the water absorption time. The hydrophobicity was studied through a contact angle of a 10 µL water droplet on the treated sample.

2.2. Recrystallization and characterization

Single crystals of GA and AG were recrystallized using a mixture of water and dimethylsulfoxide as a solvent, followed by evaporation at room temperature. The obtained single crystal was first evaluated by using a microscope with a polarizing attachment, up to 40× magnification. The crystal was sealed with epoxy adhesive to prevent degradation from moisture during the X-ray. The crystal structure of AG was obtained by single crystal X-ray diffraction (NONIUS: FR590) operated at 50 kV tube voltage and 30 mA tube current, Mo K$\alpha$ radiation with diffraction angle 2$\theta$, a scanning rate of 2$\theta$/min using the MAXUS software [35]. The crystal

![Fig. 1. H abstraction of fluorine radical SF$_6$ plasma reacting with the silk model compounds (GA and AG).](image-url)
structure of GA was obtained by single crystal X-ray diffraction (Rigaku/MSC and Rigaku Corporation. 2004) operated under the same conditions, using the Crystal Structure 3.7.0, Single Crystal Structure Analysis Software.

2.3. Quantum mechanics (QM) calculation

Molecular conformations reported from untreated singles crystals via X-ray crystallographic data were used to represent the reactants of reactions under plasma treatment. The ground state molecular minimization and transition state optimization reported here were performed using the GAUSSIAN-03 programs, while the Material Studio 4.3 program package [36,37] was used for transition state location with spin unrestricted calculations. The reaction pathway of silk model compounds treated with plasma ions was investigated using Beck-Lee Yang Parr (BLYP) functional of the Generalize Gradient Approximation (GGA) in DMol3 module of the Material Studio program (Fig. 1). The linear synchronous tran-

<table>
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Fig. 2. The crystal structures of untreated GA and AG and corresponding cell parameters using single crystal X-ray diffraction technique.

Fig. 3. (a) Initial silk model compound structures, (b) profiles of HOMO and LUMO and (c) electrostatic profiles of GA and AG.
sit (LST) and quadratic synchronous transit (QST) methods were used to study the transition state. A Fermi smearing of 0.005 Hartree was used to improve computational performance. Frequency analysis at the same level determines the nature of the stationary points and each transition state with one imaginary frequency. To fulfill the exchange term in calculations for radicals, hybrid DFT methods with high content of the orbital exchange Beck-half-and-half-Lee Yang Parr (BHandHLYP) level [38,39] of density functional theory with aug-cc-pVDZ basis set was used to calculate the energy of each stationary structure. The obtained transition state structures were confirmed through intrinsic reaction coordinate (IRC) methods with 2 Å forward and reverse distances.

Table 1

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**AG Path 1**

- Activation energy: 82.51 kcal/mol
- $H$ reaction: 36.43 kcal/mol
- $G$ reaction: 5.71 kcal/mol

**AG Path 2**

- Activation energy: 31.62 kcal/mol
- $H$ reaction: 16.40 kcal/mol
- $G$ reaction: 4.94 kcal/mol

**AG Path 3**

- Activation energy: 31.62 kcal/mol
- $H$ reaction: 16.40 kcal/mol
- $G$ reaction: 4.94 kcal/mol

**IMr1, intermediate reactant; IMp1, intermediate product; TS, transition state; P, product; HF, hydrogen fluoride; $E_a = E_{TS} - E_{IMr}$; $D_{H_{reaction}} = \sum [H(T) + E_{product}] - \sum [H(T) + E_{reactant}].**
Fig. 5. Energy profiles of six hydrogen abstraction reactions.
3. Results and discussion

3.1. Wettability effect of plasma treated silk and its model compounds

The untreated samples absorbed 10 µl water droplets immediately, while the treated samples each took time to absorb the water droplets. For each set of experimental conditions, the hydrophobicity increased after treating the sample with SF6 plasma.

For treated pellets of both silk model compounds AG and GA, silk powder had higher water repellence than AG; the contact angles of silk and AG were 131° and 61°, respectively.

3.2. Crystal structure extraction and the plasma treatment reaction mechanism

Single crystals of the simplified model compounds, GA and AG, of B. mori silk were prepared using the evaporation method described previously. The obtained single crystals were examined under a microscope with a polarizing attachment. The single crystals had an almost rectangular shape and were capable of polarizing incoming light.

The data extracted from X-ray scattering in terms of bond lengths and torsion angles are shown in Fig. 2. The crystal structure derived from the X-ray diffraction pattern of the AG compound was a monoclinic crystal system with volume of 337.27(7) Å³ and cell dimensions of \( a = 5.28 \text{ Å}, b = 11.81 \text{ Å}, c = 5.51 \text{ Å}, \) and \( \beta = 101^\circ \) in the space group \( P2_12_12_1 \). The AG crystal structure was found to be orthorhombic with a space group of \( P2_1 \) with a volume of 695.3(5) Å³ and cell dimensions of \( a = 9.68 \text{ Å}, b = 7.53 \text{ Å}, c = 9.53 \text{ Å} \).

The minimized molecular structures of the reactants for GA and AG from untreated single crystal X-ray crystallographic data in a trans conformation of the model compound were essentially the same as in a \( \beta \)-pleated conformation of protein \([11,16]\). Considering LUMO and the electrostatic potential energy profile (Fig. 3) of the model compounds, an F radical in the SF6 plasma should react with an unoccupied orbital at the methyl group of Alanine part of silk model compounds with partial positive charges.

To investigate the reaction mechanisms, the locations of stationary points were characterized by vibrational frequencies; all reactants, products, and intermediates have real frequencies, and the TSs have only one imaginary frequency. The nature of the TSs has been confirmed by the mode of the imaginary frequency and by IRC calculations.

The potential energy barrier of the AG/GA + F reaction calculated at the UBHandHLYP/aug-cc-pVDZ level is presented in Table 1, which lists total energies of reactants, TS, and reaction products, as well as the activation energy \( (E_a) \). Here, we focus on the reactions of the fluorine radicals reacting with GA and AG. The abbreviations of species corresponding to the minimal transition states in Table 1 are as follows: paths 1–3 indicate reactions at three different carbon atoms (Fig. 1) of AG and GA, respectively.

A summary of all ZPE corrected energies is given in Table 1. The table presents the calculated TS values and ZPE, as well as some geometric parameters of molecules optimized using UBHandHLYP methods (Fig. 4). The extracted crystal structure shown in Fig. 2 for GA has torsion angles of \(-164^\circ, -173^\circ, -78^\circ, 150^\circ\). Similarly, in AG the torsion angles are \(-162^\circ, 173^\circ, 71^\circ, 154^\circ\). The imaginary frequency values at each TS geometry indicated stretching motion corresponding to plausible bond breaking. The imaginary frequency at the TS, corresponding to an antisymmetric motion of the bridging hydrogen atom along the C–H–F axis, are 2073, 2157 and 1627 cm\(^{-1}\) for TS of the GA carbon atom and 1444, 2107 and 1888 cm\(^{-1}\) for TS of the AG carbon atom at three different carbon atoms. In Fig. 5, TS2 and TS3 of GA had energy lower than the reactants while simultaneously having a higher energy than the IMr. Similarly, TS3 of AG not only had lower energy than the reactants, but also had a higher energy than the IMr; thus the activation energy was calculated from transition state and IMr. From the previous discussion, the activation energies of each hydrogen abstraction of GA reacting with the fluorine radicals were 31.62, 16.40 and 4.94 kcal/mol for the TS1, TS2 and TS3 respectively. The corresponding reaction energies of the fluorine radical and AG were 82.51, 36.43 and 5.71 kcal/mol for TS1, TS2, and TS3. The coordinates of TS1 for both model compounds are less favorable. Regarding the mechanism of the hydrogen abstraction from the GA molecule by fluorine radicals, the transition state structures for the fluorine radical extracting the hydrogen of the GA molecule are shown in Fig. 4. The C1–H distance of TS1 is 1.96 Å and the F–H distance is 1.65 Å. The F–H–C1 angle is 177.60°. The C2–H distance of TS2 is 1.28 Å while the C–H distance of the GA molecule is 1.087 Å long. The forming distance of H–F is 1.29 Å (0.937 Å in HF itself) and the F–H–C2 angle is 176.46°. For the TS3 structure, the C3–H distance is 1.21 Å while the remaining C–H distances are 1.098 Å long. The H–F distance is 1.29 Å in the TS3 transition state. The F–H–C3 angle is 178.27°. Similarly, for AG reacting with fluorine radicals, the breaking C1–H bond of TS1 is stretched by 2.38 Å and the forming F–H bond is 1.92 Å. The F–H–C1 angle is 156.69°. The distance of C2–H in TS5 is 2.14 Å (the C–H bond of AG molecule is 1.088 Å) while the fluorine radical to hydrogen distance is 1.60 Å and the F–H–C2 angle is 166.12°. From the TS3 structure, the distance of C3–H is 1.28 Å. The H–F distance is 1.26 Å and the F–H–C3 angle is 177.15° in the transition state. According to the calculations in Table 1 and Fig. 5, not only do the AH of path 2 and path 3 show a strongly exothermic reaction, they also have a greater Gibbs free energy than path 1. From the barrier, thermodynamic state function and transition coordinate structure, the hydrogen abstraction mostly occurs at the alanine residue.

4. Conclusions

In order to carry out quantitative analysis concerning the relationship between structure and physical properties of Thai silk, AG and GA model compounds were introduced to simulate essential features of the parent macromolecule using molecular structures obtained from X-ray crystal structure analysis. The space groups of \( P2_12_12_1 \) and \( P2_2_2_1 \) were found for AG and GA, respectively. Molecular conformations of GA and AG from untreated single crystal X-ray crystallographic data were essentially the same as from the \( \beta \)-pleated conformation of the protein. The molecular structures were used to propose a hydrogen abstraction reaction pathway utilizing density functional theory calculations. The results from the contact angle measurement and water absorption time confirmed that the wettability of both silk and its model compounds are lower after SF6 plasma treatment. According to the calculations, the lowest activation energies for the hydrogen abstraction of both GA and AG for the F radical reaction were 4.94 and 5.71 kcal/mol respectively, at the alanine residue. Therefore, the hydrogen abstraction reactions of the alanine residue should be the preferable pathway in the plasma treatment process.

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Appendix A. Supplementary data


References