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Crystal and molecular structure of muga wild silk fibres based on $[Ala\text{-}Gly]_n$ sequence using LALS technique

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X-ray diffraction pattern of muga wild silk fibres has been recorded using imaging plate system (Dip-100S). In order to identify various Bragg reflections and to compute X-ray intensities of these reflections, fibre processing software (CCP13) has been used. A molecular model is first constructed with standard bond lengths and angles using helical symmetry and layer-line spacing observed in the X-ray pattern. The model is then refined against observed X-ray data using linked atom least squares method. The crystal and molecular structure of muga wild silk fibres are compared with reported domestic and wild silk fibre. We could get good R–factor with refinement of a model having beta-pleated sheet structures formed by hydrogen bonds having antipolar – antiparallel arrangement.

Keywords: Crystal structure, Linked atom least squares, Muga silk, Silk, X-ray technique

1 Introduction

Silk produced by caterpillars other than the mulberry silk worm is said to be the wild silk. These wild silks are of many varieties based on the worm from which it is produced. These wild silks are known familiarly and prominently used in China, Europe and in few other southern parts of Asia since ancient times. Since these worms are not cultivated domestically, the scale of production is very less as compared to the mulberry silk. These cocoons have to be collected from the wild, by the time in which the pupa will be escaped by damaging the cocoons, resulting in shorter threads. So, the use of these silk for the commercial purpose is very less and rarely found. There are two classes of silk, viz mulberry (*Bombyx mori*) and non-mulberry (Tassar, Eri and Muga). *Antheraea assamensis* (*A. Assama*) is one of the wild varieties of non-mulberry silkworm, which produces muga silk. Few other wild silks are Mopani silk from South Africa, Saturniidae silk from Thailand and Assam silks (Muga, Eri and Pat) from India, Tussah silk from China and Tassar silk from India[1]. Six million people in India alone are involved in sericulture and one of the most economically important species for sericulture in India is the wild type non-mulberry silkworm (*Antheraea mylitta*)[2,3]. Muga wild silk fibre comes under the classification of semi-domesticated multivoltine silkworm *Antheraea Assamensis* family. Muga wild silk is known for its natural shimmering colour prerogative of India and the pride of Assam state. Different techniques were used by many researchers to understand the crystal and molecular structure of domestic and wild silk fibre varieties. Crystal structure of *Bombyx mori* silk fibroin was reported by Marsh *et al.*[4]. It is revealed that the silk is made up of regular arrangements of anti-parallel sheets. Amino acid sequence, basic building blocks, of silk fibres has been reported by number of researchers[5]. Refined molecular and crystal structure of silk I based on Ala-Gly peptide sequence was studied by Kenji Okuyama *et al.*[6]. Crystal structure of pure Mysore silk (PMS) was studied by Sangappa *et al.*[7]. Crystal and molecular structure of raw Bivoltine silk fibres was studies by Mahesh and Somashekar[8]. They reported the comparison of parameters with pure Mysore silk fibre and Marsh *et al.*[4] results. Crystal structure details of tassar silk fibres were reported by Parameswara *et al.*[9]. The importance of silk protein increased because of its potential use as a natural biopolymer for tissue engineering and biomedical applications. Even though it is not so familiar to the general public, it has attracted the interest of the researchers in this area who do work on the natural polymers and silks. We have previously resolved the structures of few other

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silk races and a continued attempt has been made in this work.

In the present study an attempt has been made to give emphasis on modifications in crystal and molecular structure of Muga wild silk (Indian) fibres employing X-ray diffraction data and linked atom least squares (LALS) refinement modeling, so that one can have a better perspective of structure property relation in these fibres.

2. Materials and Methods

2.1 Sample Preparation

Fresh cocoons of muga wild silk were collected from the germ plasma stock of the Department of Sericulture, University of Mysore, which were then cooked in boiling water (100°C) for 2 min and transferred to water bath at 65°C for 2 min. These cocoons were then reeled in warm water with the help of mono cocoon reeling equipment Epprouvite. The characteristic features of these fibres are that they have natural shimmering colour, luster, smoothness and high durability. These fibres were mounted on rectangular frame in *just taut* condition, which does not involve any mechanical stretching of fibres. The whole process, starting from reeling to mounting of fibres does not involve any type of mechanical deformation.

2.2 X-ray Diffraction Measurement

XRD patterns from muga wild silk fibres were recorded on an imaging plate. A rotating anode X-ray generator (ULTRA-X, RIGAKU) was operated in a normal focus mode to provide a monochromatic beam of wavelength (λ = 0.7107 Å) at 50 kV and 250 mA. Diffraction data was recorded on a disk shaped imaging plate with the sample to plate distance of 150 mm. The measurement of X-ray diffraction data was implemented by the hardware system DIP100S (MACSCIENCE). The intensity values were thereby converted into pixel data in a rectangular coordinate system. A whole area of the imaging plate (diameter ~ 200 μm) was divided into 1600×1600 pixels, each of a size of 125mm^2. For this purpose, the program available in CCP13 suite was used (CCP13, 2004). For computation, a workstation (OCTANE, version 6.5) with an operating system (IRIS 64) was used. Ivanova and Makowshi's[10] method was used for background estimation, which is more reliable and consistent. It makes use of the fact that the background of a fibre diffraction pattern is typically composed of lower spatial frequencies than the diffraction maxima. Image plate of X-ray pattern for muga fibre is given in Fig. 1.

Each diffraction spot was picked by positioning the mouse on its centre and coordinates were measured using SUN SP/2, a SUN micro system Computer Corporation Business. After determining the centre and inclination angle of the X-ray pattern, the interplanar spacing was obtained by averaging the distances between the centre of the diffraction pattern and the positions of two or four equivalent reflections. The dimensions of unit cells were determined by least squares method with the preliminary cell dimensions being obtained by a trial and error method on the computer display. We found no significant variation in the cell parameters of different silk fibres. The averaged cell parameters are a = 9.44(5) Å, b = 10.66(3) Å and c = 6.95(7) Å. The space group of the structure being $P2_12_12_1$, and has four asymmetric units in a cell each comprising two residues. The whole pattern fitting is carried out in two stages. In the first stage, X-ray diffraction pattern taken on a flat imaging plate system was transformed to reciprocal space using the specimen to film distance, rotation of image, wavelength and tilt of the system and by employing a program FTOREC, available in the software suite CCP13. In the second step, for the whole pattern fitting, we have used a program LSQINT available in CCP13 suite for which the input file is the output of FTOREC program. By inputting unit cell parameters, the space group symmetry and

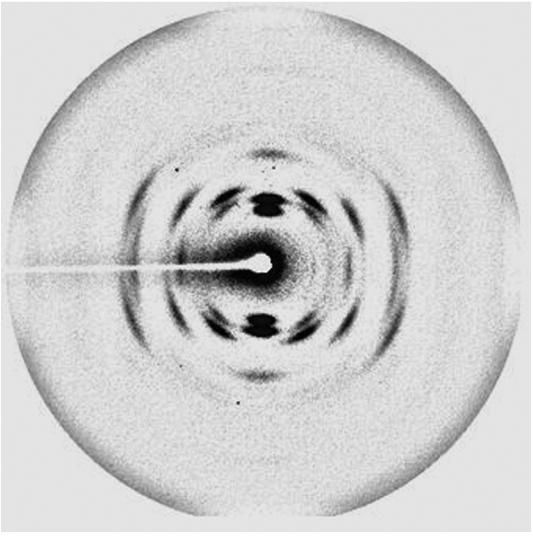

Fig. 1—Background-corrected X-ray diffraction pattern of muga wild silk fibre (multivoltine)

the profile parameters, further processing of the pattern was carried out. Using CCP13 package "XCONV" with appropriate file options suitable for DIP image system, we can simulate cylindrical image and also calculate the integrated intensities with standard deviations[11]. The output of this routine, gives simulated cylindrical pattern and computed integrated intensities. These intensities were corrected for polarization and Lorentz factors[12]. The output file which contains the integrated intensities corresponding to various (*hkl*) reflections with standard deviations are further used to determine the molecular and crystal structures of muga silk fibres.

2.3 Structure Determination

2.3.1 Molecular Model

Silk is a composite material with amino acid sequence as its primary structure. Amino acid analyses of *Antheraea* fibroins[5,12] show a uniformly high content of alanine ($[Ala]_n$). There is an option in linked-atom-least-squares (LALS) program to indicate two molecular chains by defining constrains for only one of them. Here, the geometry for Ala is for the D-Ala and not for L-Ala. The molecular conformation must satisfy both sterical and mathematical requirements. Hence, we have chosen initial φ and ψ for Ala and Gly residues to be the same as that of Marsh *et al.*[4] and the main chain was constructed with appropriate helical parameters together with bond lengths and angles.

Takahashi *et al.*[13] have shown that there are four models for the sheet structure formed by hydrogen bonds. These are: (i) polar-antiparallel [PA (1)], (ii) polar-parallel (PP), (iii) antipolar-antiparallel (AA), and (iv) antipolar-parallel (AP). In the polar model, the methyl groups of alanine residues are on one side of the sheet only. However, in the antipolar model, the methyl groups alternately point to both sides of the sheet along the hydrogen bonding direction[13]. For the positioning of molecular model in the unit cell, two additional parameters were used to define the relative axial rotation (μ) and translation (w) along the c-axis. At each stage in the modeling and refinement of the structure, we minimized the quantity Ω in the following least-squares fashion[14]. The first summation in Ω ensures the optimum agreement between the observed (F_o) and the calculated (F_c) X-ray structure amplitudes. 'w' is the weight of each reflection.

$$\Omega = \sum w\left|F_0\right| - \left|F_c\right| + S\sum_j \varepsilon_j + S\sum_k \lambda_k G_k \qquad \ldots (1)$$

The second summation over j, ensures the optimization of noncovalent interatomic (ϵ_j) parameter. Here, S is the scale factor used to adjust the overall weight of the second term with respect to the first. Third term imposes the Lagrange undetermined multipliers (λ_h) with constraints (G_h). G_h refers to constraints of the inclination of the plane

Table 1—Refined parameters of muga silk fibre with $[Ala\text{-}Gly]_n$ repeating unit

Refined parameter	Muga (*Antheraea assamensis*)	Bivoltine silk fibre	Marsh *et al.*[4]	Tassar silk fibre	Pure Mysore silk
Torsional angles (°)					
φ_{Ala}	-143.03	-158.9	-139	-141.76	-147.99
ψ_{Ala}	150.746	151.8	140	148.44	143.68
ω_{Ala}	177.169	177.4	176	179.06	178.62
φ_{Gly}	-153.878	-144.4	-139	-146.16	-144.64
ψ_{Gly}	149.865	155.9	140	138.52	146.48
ω_{Gly}	151.043	74.3	176	177.87	178.19
Eularian angles (°)					
ε_x	-75.67	-90.1	-	-86.70	-88.06
ε_y	120.66	134.6	-	126.71	128.81
ε_z	103.35	-170.9	-	-142.01	-167.87
Other parameters					
μ (°) chain-a	101.63	167.3	-	102.53	167.31
μ (°) chain-b	137.40	133.3	-	147.65	133.07
u(a); v(a); w(a)	0.7045; -3.5145; 0.5856	0.181; 0.004; 0.21	-	0.7045; -0.5145; 0.5856	0.1657; 0.0042; 0.2113
u(b); v(b); w(b)	1.5278; 1.5305; 0.3205	0.715; 0.771; 0.302	-	1.5278; 1.5305; 0.3205	0.7018;0.7712; 0.3018
Scale factor	1.039	1.28	-	1.17	2.783
Attenuation factor	-3.384	-6.245	-	-4.83	-8.755
R-factor					
R_N	0.309	0.212	-	0.330	0.168
R_w	0.325	0.205	-	0.390	0.172

described by any three atoms used to control the base-plane slope. Atomic scattering factors for calculating structure factors were obtained using the method and values given in international tables for X-ray crystallography (1974). Computations were carried out and LALS program was compiled using LINUX operating system based PC.

Table 2—Observed (F_o) and calculated (F_c) structure amplitudes for (Ala-Gly) repeating unit

Data number	h k l	Multiplicity factor	F_c	F_o
1	010	2	153.74	90.16
2	100	2	64.07	102.90
3	020	2	94.86	116.79
4	120	4	49.70	59.37
5	210	4	129.20	164.81
6	130	4	17.61	12.17
7	040	2	28.03	81.46
8	140	4	51.21	122.01
9	330	6	21.37	14.57
10	410	8	65.98	14.73
11	420	4	39.01	21.59
12	050	2	32.13	26.69
13	150	4	22.91	45.52
14	340	4	91.86	79.72
15	011	2	23.38	21.34
16	111	4	16.07	19.44
17	021	2	46.55	49.30
18	211	4	108.63	112.08
19	301	2	42.38	12.51
20	311	4	34.14	33.83
21	231	4	41.23	35.00
22	041	2	46.17	38.72
23	141	4	15.17	26.13
24	331	6	33.59	38.60
25	411	8	70.49	44.24
26	421	4	72.09	67.49
27	051	2	60.99	78.65
28	012	2	13.33	19.82
29	112	4	35.27	18.41
30	022	2	34.21	54.05
31	122	4	11.32	55.24
32	212	4	7.69	24.94
33	222	6	26.25	22.10
34	132	4	23.54	19.30
35	302	2	37.26	25.06
36	312	4	46.09	27.18
37	232	4	24.85	28.11
38	042	2	53.70	56.55
39	142	4	51.03	62.60
40	013	2	39.42	16.58
41	103	2	76.35	10.97
42	113	4	59.05	29.36
43	023	2	15.45	44.32
44	213	4	60.90	86.62

2.3.2 Molecular and Crystal Structure for Repeating Unit

The refinement was carried out for the crystal structure having two antiparallel molecules related by a 2-fold rotation axis parallel to c-axis. In order to get appropriate packing parameters, the discrepancy factors R_c (conventional R factor) and R_w (weighted R_c factor) and shortest contact between non bonded atoms were calculated. Here, R_c and R_w are defined by the following equations:

$$R = \sum \|F_o\| - \|F_c\| / \sum \|F_0\| \quad \ldots (2)$$

$$R_w = \sum W(|F_o| - |F_c|)^2 / \sum F_o^2 \quad \ldots (3)$$

A dipeptide Ala-Gly was used as chemical repeating unit for refinement. The weight of each reflection 'w' was fixed to 1.

3 Results and Discussion

The refined parameters and the final values are summarized in Table 1. Here, the azimuthal angles of the two molecules forming a sheet were also refined independently. Table 2 gives the comparison between the observed and calculated structure factors. Fractional atomic co-ordinates for (Ala-Gly) residues are given in Table 3. The crystal structure of muga

Table 3—Fractional atomic co-ordinates of Muga wild silk fibre for the repeating unit of (Ala-Gly)

Atom	Chain a			Chain b		
	X	Y	Z	X	Y	Z
Ala						
N	0.237	0.653	0.060	0.262	0.346	0.560
H_N	0.247	0.747	0.050	0.252	0.252	0.550
C_α	0.272	0.581	0.231	0.227	0.418	0.731
C_β	0.431	0.557	0.237	0.068	0.442	0.737
H_α	0.219	0.499	0.224	0.280	0.500	0.724
C^1	0.228	0.654	0.411	0.271	0.345	0.911
O	0.230	0.770	0.413	0.269	0.229	0.912
Gly						
N	0.188	0.583	0.559	0.311	0.416	1.059
H_N	0.186	0.489	0.556	0.313	0.510	1.056
C_α	0.147	0.644	0.737	0.352	0.355	1.237
$H_{\alpha1}$	0.041	0.655	0.740	0.458	0.344	1.240
$H_{\alpha2}$	0.193	0.728	0.748	0.306	0.271	1.248
C^1	0.189	0.566	-0.085	0.310	0.433	0.414
O(2)	0.183	0.464	-0.066	0.316	0.535	0.433

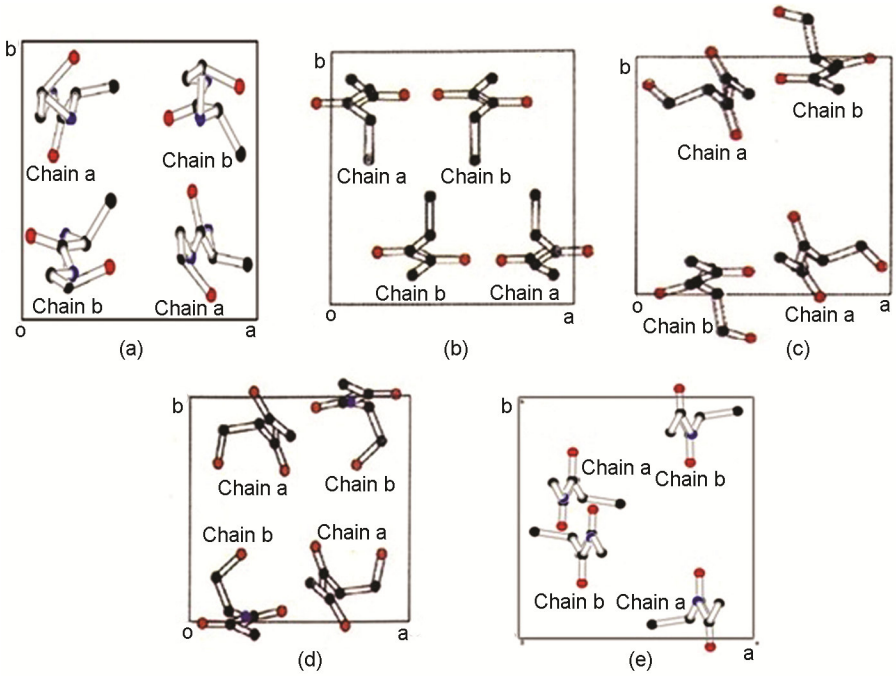

Fig. 2—Down the fibre axis projection of crystal structure of (a) muga wild silk fibre, (b) obtained by Marsh *et al*.[4] , (c) pure Mysore silk fibre, (d) Bivoltine silk fibre, and (e) Tassar wild silk fibre [red–oxygen atom, blue–nitrogen atom and black–carbon atom]

silk in raw form is shown in Fig 2. The internal rotation angles (ω) of glycine and alanine residues about N-C(=O) bonds are 151.04° and 177.16° respectively which are nearly *trans* conformation. The φ and ψ for the glycine residue are -153.87° and 149.86° respectively which are also between *skew* and *trans* conformations. The φ and ψ for the alanine residue are -143.03° and 150.74° respectively, which are in agreement with the values (φ = -146.67° and ψ = 143.00°) given by Pauling and Corey for antiparallel-chain model[15,16]. The torsional angles and Eulerian angles are the same for both chain-a and chain-b as they are symmetric.

Crystal structure down the *c*-axis (i.e. fibre axis) of muga wild silk fibre [Fig. 2(a)] indicates that the chains 2 & 2' are shared and twisted along the fibre axis. The steriochemical energy which is represented by σ is found to be 2.37E+04 kcals. Here, the σ is given by the sum of second term of the Eq. (1)[6]. We observe that the molecular modification is essentially same as β-pleated structure with antipolar-antiparallel arrangements formed by hydrogen bonds. In a unit cell, the position of the C=O group of chain along c-axis is oriented in one direction at one position and in opposite direction in another position. Such an arrangement results in a dipole, which stabilizes the crystal lattice of muga wild silk fibre.

4 Conclusion

Crystal and molecule structure of muga wild silk fibre, belonging to *Antheraea assamensis* family was studied by X-ray diffraction data and employing Linked-Atom-Least-Squares method. Here we find four molecular chains are contained in the rectangular unit cell with parameters a = 9.44 Å, b = 10.64 Å and c = 6.95 Å and the space group being $P2_12_12_1$. On comparing the crystal structure of silk II proposed by Marsh *et al.*[4], it is observed that there is certain twist and shearing of the molecular chain along the fibre axis in muga wild silk fibre. From the obtained values of torsional angles, Eulerian angles and other parameters, we conclude that in muga wild silk fibre, the sheet structures formed by hydrogen bonds assume the antipolar-antiparallel arrangement, which is in conformity with the earlier reported results. It is observed that the stabilization of the model comes from alternate arrangement of bonds C=O in the neighboring chains which results in a net small dipole moment.

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