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Study of the degradation mechanism of Chinese historic silk (*Bombyx mori*) for the purpose of conservation

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ABSTRACT

Chinese historic silk fabrics dated from the Warring States Period (B.C. 475–B.C. 221), the Han Dynasty (B.C. 202–A.D. 220) and the Ming Dynasty (A.D. 1368–A.D. 1644) as well as artificial aged silk specimens were examined by SEM, TEM, amino acid analysis, FTIR, WAXD, SAED and DSC to investigate the degradation mechanism for the purpose of conservation. The variations of the relative concentrations of Gly and Ala and the crystallinity revealed that the degradation mainly occurred at the amorphous region at the initial degradation stage; both the amorphous and the crystalline regions were degraded at the same speed at the late degradation stage. Combined with the fact that the mechanical strength decreased over the degradation process, it could be concluded that the degradation of both the amorphous and the crystalline regions contributed to the decrease of the mechanical strength. During the degradation process, the morphology of the cross-section of the silk fiber was found to change from stacking particles to lamellar sheets, with decreasing size of segments of silk fibril. The change of morphology and degree of orientation during the degradation process exhibited correlations with the change of secondary structure, which was greatly affected by aging method. The change of thermal stability exhibited correlations.

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1. Introduction

Silk culture is an important yet vulnerable part of Chinese cultural heritage. However, due to the 100% protein composition, silk is not resistant to environment and is easily attacked by various factors including water, microorganism, light and heat [1-5]. As a result, most of the historic silk textiles and artifacts lost their mechanical strength and became more or less brittle after undergoing long time of degradation (Fig. 1). In order to determine suitable approaches to the treatment, display and storage of historic silk textiles and artifacts, it is important to be able to characterize the physical and chemical state of the silk fibers and understand the degradation process.

Raw silk is a proteinaceous fiber consists of two filaments of protein, fibroin, with high crystallinity and strong preferred orientation, which are bound together by a principally amorphous protein, sericin [6] which can be removed by degumming. Most historic silks and artifacts are made of degummed silk i.e. fibroin and therefore their degradation is in fact the degradation of fibroin. Fibroin is composited mostly of the amino acids alanine, serine and glycine [7] which fold into antiparallel β -sheets as repeated motifs (– Gly-Ala-Gly-Ala-Gly-Ser-) and aggregate into crystallites which are considered to be responsible for the excellent mechanical property of silk because of their highly ordered structure. The X-ray examinations show that the crystalline region has a rectangular unit cell containing four molecules, with space group P21 and parameters, a (interchain direction) = 9.44 Å, b (intersheet direction) = 8.95 Å and c (fiber axis) = 7.00 Å [8–10]. The unit cell is composed of stacking of two antipolar-antiparallel sheet structures with different orientations. The crystallites are uniformly embedded in an amorphous matrix which is rich in residues with bulky, polar sidechains. The amorphous region is characterized by tyrosine residues and is thought to adopt distorted β -sheets, distorted β -turns and 3₁helices [11,12] structures, loose structures that are considered to confer the silk elastic property. Due to the complex structure, it is challenging to understand the degradation process of silk fibroin.

Up to now, degradation behavior of regenerated fibroin has been widely researched [13–17], while research about the degradation of



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Fig. 1. Photographs of fresh and historic specimens. (a) Fresh silk with smooth surface and bright color. (b) Ming silk (~500 year) with colored and mildew contamination. (c) Han silk (~2020 year) broke into pieces with browny color and brittle quality. (d) Zhan silk (~2360 year) with soil contamination and brittle quality. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

natural fibroin fibers is limited. Peacock [1] used light microscope and scanning electron microscope to evaluate the influence of soil burial and water on the morphology of silk fibers, which were proved to be different. Zhang et al. proposed that viscosity [18] could be used as an indicator of residual tensile strength and Asp/Gly molar ratio [2] and Tyr content was useful for gauging the conservation state of silk. Garside [6] related certain measurable spectroscopic, chromatographic and chemical signatures to the physical deterioration of silk and found out that, during the aging process, the amorphous region broke down and the crystallites progressively lost their strong alignment with the fiber axis but stayed intact until the ultimate stage. However, knowledge about degradation of natural silk fibroin fiber is still far from being exhaustive at present due to the complicated and precise hierarchical structure.

In the present study, historic silk fragments with increasing burial time from the Ming Dynasty (\sim 500 y) to the Warring States Period (\sim 2350 y) were investigated, trying to find out the real degradation process of silk fibroin from the aspect of hierarchical structural change and the inherent correlation by comparing the historic silk specimens with the fresh and the artificial aged ones. Amino acid analysis, scanning electron microscope (SEM), transmission electron microscope (TEM), Fourier-transform infrared spectroscope (FTIR), X-ray diffraction (XRD), selecting area electronic diffraction (SAED) and thermal analysis were used to characterize the structural changes. The results were thought to be helpful in the other researches which involved fibroin degradation.

2. Experimental

2.1. Materials

Plain weaved modern natural silk textiles were purchased from Hubei natural Science and Technology Co., Ltd. Historical silk fabrics, excavated from Jingzhou City, Hubei Province, China and dated back to the Ming Dynasty (A.D. 1368–A.D. 1644), the Han Dynasty (B.C. 202–A.D. 220) and the Warring States Period (B.C. 475–B.C. 221) respectively, were supplied by Jingzhou Preservation Center of Culture Relics (Jingzhou, China). In this paper, they were denoted as Ming, Han and Zhan respectively. All the historic specimens were carefully cleaned with water, acetic ester and petroleum ether sequentially to remove the contamination before observation.

2.2. Preparation of artificial aged modern silk samples

Hydrolysis, heat and light aged specimens were prepared to mimic the possible influence of environment on historic specimens and assist the understanding of degradation progress. The mechanical properties of artificial aged specimens were designed to be close to those of historic specimens.

2.2.1. Alkaline hydrolysis aging

Fresh specimens were immersed in 1 wt% NaOH solution for 5 d and the temperature was kept at 50 $^\circ\text{C}.$

2.2.2. Heat aging

Fresh specimens were placed in the thermostatic chamber and the temperature was kept at 150 $^{\circ}\text{C}$ for 10 d.

2.2.3. Ultraviolet (UV) light aging

Fresh specimens were exposed to UV lamps transmitting at 254 nm for 10 d at 50 \pm 10 °C and the relative humidity was controlled at 55 \pm 5 RH%. Ozone flow (300 µg/h) was delivered 0.5 h for every 2 h. Specimens were turned over every 1 h.

2.3. Characterization

Scanning electron microscope (SEM) images of samples were observed with a Quanta 200 Scanning Electron Microscope (FEI, USA) at 30 keV accelerating voltage and high magnified images were observed with a Quanta FEG 450 (FEI, USA) at 15 keV accelerating voltage. Specimens were mounted on an aluminum stub and sputtered with aurum before observed. Brittle fractures of the fresh and the Ming silk were obtained with liquid nitrogen. TEM images and energy-dispersive X-ray spectra were observed with a 200 keV IEOL 2000FX (Japan) equipped with EDS. Sections of 60–90 nm thickness were cut with a diamond knife on an LKB V Microtome, and then placed on 240 mesh copper grids. Selected area electron diffraction (SAED) was also performed using a 200 keV JEOL 2000FX (Japan). Specimens were grinded in an agate mortar, ultrasonic dispersed in water and then placed on 400 mesh copper grids. Amino acid composition analysis was performed by a HITACHI-835 Amino Acid Analyzer. Samples were hydrolyzed with 6 M HCl at 110 °C for 24 h. The hydrolyzate was diluted with water to 25 mg/mL and the diluted solution was analyzed. FTIR spectra were recorded using a Fourier-transform infrared spectroscopy (NICOLET 5700, USA). Spectra over the range

4000–400 cm⁻¹ were collected with a resolution of 4 cm⁻¹. X-ray diffraction (XRD) of samples was performed by an X' pert Powder diffractometer (PANalytical, Holland) with Cu K α radiation from a source operated at 40 kV and 40 mA. Differential scanning calorimetry (DSC) was performed on samples under nitrogen atmosphere. Samples were heated in Diamond TG-DTA analyzer until completely decomposed (~600 °C) in an aluminum crucible at a heating rate of 30 °C/min. The flow rate of nitrogen gas was 30 mL/min.

3. Results and discussion

3.1. Morphology

Physical status of silk fibers can be directly manifested by morphology. Surfaces of fresh and historic silk specimens were observed by SEM. It could be seen that fresh silk fiber had a diameter of about 10 μ m (Fig. 2a). The surface was smooth and clean,



Fig. 2. SEM images of the surfaces of (a) fresh, (b) Ming, (c) Han and (d) Zhan silk, scale bar 10 µm. TEM images of the cross-sections of (e) fresh, (f) Ming, (g) Han and (h) Zhan silk, scale bar 5 µm. SEM images of magnified cross-sections of (i) fresh, (j) Ming, (k) Han (l) Zhan, (m) hydrolysis aged, (n) heat aged and (o) light aged silk, scale bar 500 nm. (p) Typical energy-dispersive X-ray spectrum of the surface of historic silk.

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Amino acid	concentration of each specimen	(g/100 g).

	Fresh	Ming	Han	Zhan	Hydrolysis	Heat	Light
Asp	2.07	0.93	0.56	0.73	0.48	0.58	1.18
Thr	0.93	0.62	0.45	0.40	0.42	0.50	0.63
Ser	14.62	12.46	11.42	7.29	13.62	13.81	10.96
Glu	1.74	1.02	0.81	0.76	0.49	0.64	1.32
Pro	0.66	0.42	0.43	0.41	0.42	0.24	_
Gly	35.24	35.97	32.43	22.38	40.24	39.83	31.09
Ala	27.68	27.64	24.96	17.17	31.08	30.56	26.69
Val	2.62	2.42	2.10	1.63	2.34	2.32	2.19
Met	0.06	0.04	0.03	0.03	0.04	0.02	_
Ile	0.88	0.54	0.50	0.50	0.38	0.43	0.54
Leu	0.86	0.44	0.50	0.51	0.28	0.25	0.38
Tyr	10.52	8.99	8.07	5.80	9.11	9.46	1.66
Phe	1.24	0.78	0.52	0.53	0.52	0.50	0.90
Lys	0.52	0.14	0.26	0.18	0.10	0.09	0.13
His	0.38	0.16	0.18	0.12	0.42	0.36	0.04
Arg	1.01	0.5	0.38	0.49	0.42	0.34	0.67
Total	101.0	93.1	84.60	58.9	100.4	100.0	79.6

with horizontal lines on its surface along the axis. All the historic silk specimens showed roughness (Fig. 2b–d) and the extent increased with the increasing burial time. Especially, the Zhan silk was enwrapped by irregular sheet structures which could not be removed by washing with water, acetic ester and petroleum ether and were proved to be composed of elements Si, S, Cl, K and Ca by the energy-dispersive X-ray spectra (EDS) (Fig. 2p). In fact, these elements were also observed in the other historic silks more or less and were thought to originate from the burial environment.

Cross-sections of fresh and historic silk specimens were also observed. As the TEM images of the cross-sections shown (Fig. 2e– h), fresh silk showed an intact triangle cross-section while historic specimens showed increasing strip cracks, which were produced in the microtoming process, with the increasing burial time, suggesting that the loss of mechanical strength i.e. the deterioration extent had a positive correlation with the burial time. To further investigate the morphological changes inside the fibers, high magnified images of cross-sections were observed. As Fig. 2i shown, the cross-section of fresh silk was actually composed of compactly stacked particles with sizes of about 50–70 nm. Similar particles were also found in other Refs. [19,20]. They were considered to be segments of silk fibril and composed of β -sheets (crystalline domains) connected by non- β -structure (noncrystalline domains). With the increasing of burial time, the size of the particles became smaller and the stacking of the particles became looser. In the case of the Zhan silk, particle structure almost disappeared and lamellar sheets were shown instead. To clarify the influence of different aging methods, cross-sections of artificial aged specimens were also examined (Fig. 2m–o). Light aged specimen showed a similar cross-section with that of the Ming silk and the hydrolysis and heat aged specimens showed similar lamellar morphology with that of the Zhan silk.

3.2. Primary structure

To understand the degradation progress, investigation into the compositional change is required. As listed in Table 1, all the total amino acid concentrations of fresh, hydrolysis aged and heat aged silk specimens were 100 g/100 g, indicative of 100% amino acid composition. For all the historic specimens and light aged specimens, the total amino acid concentrations decreased, which was caused by the residues that could not be hydrolyzed by 6 M HCl before amino acid composition analysis. The FTIR spectra and the normalized EDS results of the residues (Fig. 3) were collected to study the structural change. As the aging history of historic samples was not clear, we proposed a possible way of assignments of the absorption bands mainly based on the light aged sample with the assistance of EDS result (Fig. 3b). It could be seen that the characteristic amide absorption bands of fibroin (which will be discussed later) did not appear. The absorption band at 1705 cm⁻¹ was assigned to stretching of C=O [21,22], the bands at 1630 cm⁻¹(asymmetric stretching of NO₂ and stretching of C=0) and 1384 cm⁻¹ (symmetric stretching of NO₂) were assigned to nitro group [21,23,24] and the broad band at 1089 cm⁻¹ might come from the stretching of sulfoxide group [25] and asymmetric stretching of C–O of ozone oxidization product [26] (only for the light aged sample as the light aging was performed with the existence of ozone in this study). The assignments were supported by the fact that reactive oxygen (ROS), which could be generated by UV



Fig. 3. a) FTIR spectra and b) EDS results of the hydrolysis residues of light aged, Ming, Han and Zhan silk.

 Table 2

 Relative amino acid concentration of each specimen (g/100 g).

	Fresh	Ming	Han	Zhan	Hydrolysis	Heat	Light
Asp	2.07	1.00	0.67	1.23	0.48	0.58	1.48
Thr	0.93	0.67	0.53	0.68	0.42	0.50	0.79
Ser	14.62	13.38	13.49	12.37	13.62	13.81	13.77
Glu	1.74	1.10	0.96	1.29	0.49	0.64	1.66
Pro	0.66	0.45	0.50	0.70	0.42	0.24	_
Gly	35.24	38.64	38.93	38.00	40.24	39.83	39.06
Ala	27.68	29.69	30.09	29.16	31.08	30.56	33.53
Val	2.62	2.60	2.48	2.77	2.34	2.32	2.75
Met	0.06	0.043	0.030	0.051	0.04	0.02	-
Ile	0.88	0.58	0.59	0.84	0.38	0.43	0.68
Leu	0.86	0.47	0.59	0.86	0.28	0.25	0.48
Tyr	10.52	9.66	9.54	9.84	9.11	9.46	2.09
Phe	1.24	0.84	0.61	0.90	0.52	0.50	1.13
Lys	0.52	0.15	0.31	0.31	0.10	0.09	0.16
His	0.38	0.17	0.21	0.20	0.42	0.36	0.05
Arg	1.01	0.54	0.45	0.84	0.42	0.34	0.84

irradiation and ozone, mediated oxidation of proteins could lead to nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and conversion of some amino acid residues to carbonyl derivatives [25,27]. The absorption band at 1515 cm⁻¹ might be assigned to Tyr side chain [22,28–30], suggesting that Tyr side chain is susceptible to oxidative degradation. Historic silk samples showed carboxyl, carbonyl, nitro and sulfoxide groups, suggesting that historic samples might have suffered oxidative damage. Besides, all the historic silk samples showed bands at 1090 cm⁻¹ (mainly came from Si–O asymmetric stretching with minor contributions from the stretching of sulfoxide), 798 cm⁻¹ (symmetric stretching of Si–O–Si bridges) and 466 cm⁻¹ (bending of O–Si–O linkage) which were assigned to SiO₂ [31–33] and probably originated from the burial environment. As shown in the EDS spectra (Fig. 3b), the SiO₂ content increased with the increasing burial time, indicative of the accumulation of SiO₂ on the silk fiber during the burial time. The decrease of total amino acid concentration was not found in the hydrolysis and the heat aged specimens, suggesting that this kind of degradation was not induced by hydrolysis and heat aging.

For the hydrolysis and the heat aged specimens, the concentrations of all the amino acids decreased except Gly and Ala. Combined with the observed weight loss percentage of 37% and 39% of the hydrolysis and heat aged specimen respectively, which was attributed to the removal of the small molecular weight hydrolyzates and the gaseous products of thermal degradation (such as CO₂ and NH₃), the increased concentrations of Gly and Ala were considered as the result of the comparably rapid loss of the other amino acids. The increase of the Gly and Ala concentrations was also found in the historic and the light aged specimens when calculating the concentration of each amino acid into relative concentration (Table 2) versus total amino acid concentration as shown in Fig. 4a and b. For the light aged sample, the increase of relative concentrations of Gly and Ala was attributed to the comparably rapid loss of the other amino acids caused by both the hydrolysis residue and the weight loss ($\sim 20\%$) as a result of the removal of the gaseous product (aldehvde, NH₃ and CO₂) of ROS oxidation [27,34]. The comparably rapid loss of the other amino



Fig. 4. Relative content variations of Gly, Ala, Ser, Tyr of (a) historic silk group and (b) artificial aged group and relative content of the other amino acid of fibroin of (c) historic silk group and (d) artificial aged group.

acids could be explained as following: Gly, Ala and Ser formed the crystalline region of the fibroin fiber as repeated motifs (-Gly-Ala-Gly-Ala-Gly-Ser-)₈ and the rest amino acids mainly located in the amorphous region. The poorly ordered and loose amorphous region degraded more rapidly than the ordered and compactly stacked crystalline region and made the relative concentrations of Gly and Ala increase. At the same time, it should be noticed that the concentrations of Gly and Ala of the Ming silk were nearly the same as those of the fresh silk, indicating that the crystalline region of the Ming silk was almost intact. It meant that the degradation of the Ming silk mainly occurred at the amorphous region. It was consistent with the literature published previously [6].

Besides, it was worth noticing that, compared to the fresh silk, the relative concentrations of Gly and Ala of the Ming increased dramatically, from 35.24 g/100 g to 38.64 g/100 g and 27.68 g/100 g to 29.69 g/100 g respectively, while the relative concentrations of Gly and Ala of the Han and the Zhan changed slightly compared to the Ming silk, maintaining at around 38 g/100 g and 30 g/100 g respectively. Considering the respective degradation time of the historic silk specimens, \sim 500 y (the Ming), 2000 y (the Han) and 2350 y (the Zhan) respectively, it could be concluded that, only the amorphous region was degraded at the initial degradation stage and the crystalline region remained intact; at the late degradation stage, both the crystalline and the amorphous region were degraded at nearly the same speed. The relative concentrations of Gly and Ala of the artificial aged group were a little higher than those of historic group, suggesting that the amorphous region of the artificial aged specimens degraded more seriously.

3.3. Secondary structure

Physical and chemical properties of silk fibroin were closely related to the secondary structures. FTIR spectroscopy was sensitive to the secondary structure change of fibroin protein and the assignments of absorption bands have been widely researched [11,28–30,35]. Absorption bands at region 1640–1660 cm⁻¹ (amide I, C=O stretching) and 1240–1260 cm⁻¹ (amide III, C–N stretching) and N-H deformation) were assigned to α -helix/random coil structure and absorption bands at region 1668–1670 cm⁻¹, 1600– 1640 cm^{-1} (amide I) and 1200–1240 cm^{-1} (amide III) were assigned to β-sheet structure. The characteristic absorption bands of all the samples were shown in Fig. 5 and Table 3. It could be seen that the fresh silk had a typical β -sheet structure with absorption peaks at 1670 and 1234 cm⁻¹. The Han and the hydrolysis aged specimens showed different β -sheet structure from that of the fresh silk, with absorption peaks at 1630 and 1231 cm⁻¹. It suggested that the Han silk probably had hydrolysis aging history and its secondary structure was different from that of the fresh silk at molecular level although it also adopted β -sheet structure. The newly appeared peaks at 1700 cm⁻¹ of the Han and the hydrolysis aged specimens were assigned to β -turn conformation of the hairpin-folded antiparallel β -sheet structure [11,28–30,36–38], which was formed during the conformation transition process. The peak located at 1262 cm⁻¹ in the spectra of the Han and the Zhan silk was also assigned to unordered or less ordered β-turn structure [11,28–30,37]. The Zhan silk had a similar spectrum with that of the Han except that the amide I peak located at 1648 cm⁻¹ instead of 1632 cm⁻¹, indicative of lower β -sheet conformation fraction than the Han silk. The Ming, the heat aged and the light aged specimens displayed basically *a*-helix/random coil structure with peaks at 1655 and 1235 cm⁻¹. In addition, the amide II region, assigned to the ring CC stretching and CH bending mode of the Tyr side chain [28–30], of the specimens with α -helix/random coil structure (including the Ming, the heat and the light aged silk) appeared at



Fig. 5. FTIR spectra of (a) fresh, (b) Ming, (c) Han, (d) Zhan, (e) hydrolysis aged, (f) heat aged and (g) light aged silk.

around 1530 cm⁻¹ instead of 1520 cm⁻¹ of the specimens with β -sheet structure (including the fresh, the Han, the Zhan and the hydrolysis aged silk), reflecting the increase of conformational freedom [33]. Different conformations of the historic silk specimens might suggest different aging histories.

3.4. Crystal structure

The crystal region of silk fibroin was mainly composed of compactly aligned antiparallel *β*-sheet structure and played a significant role in determining the physical and chemical properties of fibroin such as tensile strength, solubility, orientation and so on. Crystal structure of specimens was examined by WAXD and the diffractograms were shown in Fig. 6. The fresh silk was characterized by a broad and strong diffraction peak centered at 20.5° and a minor peak at 8.8°. The reflections were indexed following Rössle [37] and the peak at 20.5° was assigned to a multi peak arising from (020), (210) and short range ordered structure. As displayed in Fig. 6, changes of the diffractograms over degradation process mainly occurred at the multi peak. The intensity of the multi peak of the historic specimens decreased over the aging process whereas the intensity of the Ming and the Han silk were higher than that of the fresh silk. When the aging time increased to \sim 2350 year (the Zhan silk), the intensity considerably decreased compared to that of the Han silk. Combined with the results obtained in the amino acid analysis, it could be explained as following: at the initial stage of degradation, degradation mainly occurred at the amorphous region, leaving the crystalline region nearly intact; the loss of disorder made the crystallinity increase. As deterioration progressed, degradation occurred at both the amorphous and the crystalline region, which loosened the compact

Table 3				
Locations of the	characteristic absorption	bands	in FTIR	spectra

	Amide I (cm ⁻¹)	Amide II (cm ⁻¹)	Amide III (cm ⁻¹)
Fresh	1670.2	1519.6	1234.1
Ming	1655.2	1534.5	1234.1
Han	1632.3	1518.4	1231.0, 1261.6
Zhan	1647.9	1518.6	1233.7, 1261.6
Hydrolysis	1632.3	1520.2	1233.7
Heat	1655.0	1528.6	1235.8
Light	1655.1	1531.5	1236.2



Fig. 6. XRD diffractograms of (a) historic, (b) hydrolysis aged, (c) heat aged and (d) light aged silk groups.

alignment of β -sheets and led to the decrease in crystallinity. At the late stage of degradation, the well alignment of not only β -sheet structure but also short range ordered structure was seriously broken, maybe accompanied by the scission of polypeptide chain and conformation transition, thus the crystallinity dramatically decreased. In addition, the intense peaks appeared at 21.0° and 26.6° of the Zhan silk were assigned to SiO₂ [39,40] whose existence could be proved by the FTIR spectra (Fig. 3a) and was considered to originate from the burial environment and could not be removed easily. For the artificial aged group, the intensity of the multi peaks of all the three specimens was close to that of the Zhan silk, indicating that hydrolysis, heat and light aging could seriously degrade the crystalline region.

In order to investigate the crystalline structural changes in more details, SAED (selected area electron diffraction) was performed. The diffraction patterns could indicate the ordering within a material, with the intensity, spacing and orientation of diffraction spots signaling the extent and arrangement of crystalline components: amorphous regions produce halos [7]. As shown in Fig. 7, as the diffraction patterns were obtained from the ground fragments dispersed in water instead of single fiber and the fibroin protein degraded rapidly in the electronic beams, the observed pattern of fresh silk fiber was different from the typical texture reported by Marsh [41] and Warwicker [8] and thus complicated to index, but still exhibited sharp rings constituted of discontinuous spots and symmetrical diffraction arcs, which indicated the strong alignment of β -sheet crystallites along the fiber axis. Compared to fresh silk, the symmetrical arcs disappeared and the discontinuous spots spread into continuous rings in the patterns of all the historic and artificial aged specimens, suggesting the weakening of strong orientation. However, the detailed diffraction patterns varied. Specimens with β -sheet structure, including the Han, the Zhan and the hydrolysis aged specimens, retained relatively more rings and the shapes were relatively sharper and well-defined, implying better β -sheet conformation. What was unexpected was that the pattern of the Han silk was relatively less defined than those of the other two specimens despite higher crystallinity. This could be attributed to the different degradation levels of the amorphous region. The more seriously degraded amorphous regions of the Zhan and the hydrolysis aged silk, indicated by the higher relative concentrations of Gly and Ala, gave more mobility to the polypeptide chain and allowed its better free adjustment and tighter formation of β -sheet conformation. Oppositely, specimens with α helix/random structures, including the Ming, the heat and the light aged specimens, demonstrated obscure rings and amorphous halo, representative of poor orientation and disordered alignment. The disordered extent showed a negative correlation with the crystallinity. Moreover, for the artificial aged group, it could be seen that their diffraction patterns differed a lot in spite of similar crystallinity, indicating that hydrolysis, heat and light aging degraded the crystalline region in different ways and the crystalline structure was largely affected by aging history.



Fig. 7. SAED diffraction patterns of (a) fresh, (b) Ming, (c) Han, (d) Zhan, (e) hydrolysis aged, (f) heat aged and (g) light aged silk, scale bar 5.1/nm.

3.5. Thermal properties

Thermal analysis was performed to confirm the structural changes. The DSC curves were shown in Fig. 8. The thermal decomposition of all the specimens took place at a single step and the DSC curve of fresh silk was characterized by a single predominant endothermic transition at around 327 °C. attributed to the thermal decomposition of the silk fibroin with orientated β -sheet conformation [42-44]. The native fibroin fiber showed much higher decomposition temperature than that of regenerated fibroin film (270–290 °C [29,30,43,44]) due to its highly oriented β -sheet conformation. As the decomposition temperature was highly dependent on crystallinity and molecular orientation [44]. The higher the crystallinity and the degree of molecular orientation were, the higher the decomposition temperature. For the same reason, the Ming and the light aged silk displayed lower decomposition temperature at around 325 °C and 300 °C respectively, corresponding to silk I structure. It was noticed that the decomposition temperature of the Ming silk was close to that of fresh silk although the degree of orientation was lower (Fig. 7b). This was considered as the integrated result of the higher crystallinity and the lower degree of orientation of the Ming silk. The Han, the Zhan and the hydrolysis aged specimens displayed transitions at round 336.9 °C, 339.6 °C and 336.5 °C respectively, corresponding to silk II



Fig. 8. DSC curves of (a) historic silk group and (b) artificial aged group.

structure. The higher transitions than that of the fresh silk were considered as the results of the seriously degraded amorphous region and the self adjustment of polypeptide chain to tight β -sheet structure. In the case of the heat aged silk, it showed a transition at 336.5 °C despite silk I structure. This might be explained by its diffraction pattern. As shown in Fig. 7f, fine crystal faces were visible in the pattern although amorphous halo predominated, implying the presence of ordered structure which improved the thermal stability. In a word, it could be found that the thermal stability of fibroin was closely related to the secondary structure and the degree of orientation of crystallites.

3.6. Correlation between mechanical property and degradation process

As mechanical properties have great influence on the display and the storage of historic silk fabrics, the correlation between the mechanical properties of silk and the degradation process was discussed. As displayed in Fig. 2e-h, the mechanical strength decreased over the whole degradation process no matter that the degradation occurred at only amorphous region or at both amorphous and crystalline regions, it could be concluded that both the degradation of crystalline and amorphous region contributed to the weakening of mechanical strength. This could be explained as follows: Fibroin could be considered as a two-phase material. The amorphous region acted as soft and flexible matrix and the crystalline region acted as stiff and oriented reinforcement. The loss of amorphous region meant the weakening of the ability of stress conducting and buffering. The decrease of the crystallinity and the degree of orientation of the crystalline region meant the decrease of the load bearing capacity.

4. Conclusions

Based on the integrated characterization of historic and artificial aged silk specimens, a probable degradation process of silk fibroin was proposed. The degradation mainly occurred at the amorphous region at the initial stage, which made both the relative concentrations of Gly and Ala and the crystallinity increase. As degradation progressed, both the crystalline and the amorphous regions were degraded at the same speed. As a result, the relative concentrations of Gly and Ala nearly remained constant, at around 38 g/100 g and 30 g/100 g respectively, and the crystallinity decreased. At the late stage, the crystallinity further decreased due to the further degradation of β-sheet and short range ordered structure. The degradation of both the crystalline and the amorphous region contributed to the loss of mechanical strength of aged silk. On the other hand, the degradation process of silk was accompanied by the changes of internal morphology, secondary structure, degree of orientation and thermal stability. The morphology of the cross-sections changed from stacking particles to lamellar sheets, with decreasing size of fibril segments. The secondary structure was greatly affected by aging methods. The degree of orientation reduced generally and the change pattern exhibited great correlation with the secondary structure. The thermal stability changed irregularly and the change pattern exhibited correlation with the secondary structure and the degree of orientation. The specific correlations still needed further systematic research.

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