

INFRARED STUDIES OF SILK FIBROIN

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INTRODUCTION.

As is well known, we have to discriminate between *Bombyx mori* and Wild silk fibroin, that is, there are much different import tax between the two. The basic structure of silk fibroin was examined by many worker on the basis of characterical powder X - ray diffraction patterns and infrared.

But there were not good information, because it was very difficult to make sample for infrared studies.

The most striking differences are the relative amount of glycine and alanine. For the proteins such as silk, which consists largely of the residues of the small amino acids, glycine and alanine, has advantages.

The molecular weigh is high and there are apparently no cross links.

So the properties may be expect the reasonable the synthetic polypeptides, just like whose configuration much is already known.

In particular, the amount of glycine in wild silk is contrast to the smaller percentage of glycine, the percentage of alanine is much larger in wild silk than in *bombyx mori*.

According to Lucas, Shaw and Smith, wild silk contains approximately 39 glycine,50 alanine out of a total of 100 residues, whereas *bombyx mori* silk has 41 glycine,28 alanine, with a correspondingly higher proportion of bulky side chains than is found in wild silk.

It seems reasonable to expect that the structual differences between the two silk, as evidence by the differences in their infrared and X - ray patterns, are relative in a simple way to whose striking differences in their chemical com - position. In another words, two fibroins shows how dominant is the effect of the glycine and alanine residues.

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EXPERIMENT

The bombyx mori, wild silk and poly - L - alanine in this study was polymers obtained from the sericultural experiment station and polyglycine polymer got from University of Michigan.

Silk fibroin were obtained from the general extraction and refined.

Silk fibroin sample which were examined were prepared by casting a film from the 1% trifluoroacetic acid solution on polyethylen film and AGCL plate, after that, we teared off a film from polyethylene and AGCL plate, and spectra were obtained with a NIHONBUNKO IR - S double beam and HITACHI 225 double - beam grating spectrophotometer. We had also investigated the film which it rolled and treatment of alcohol and water.

To study the structual differences between the two silks, that is, bombyx mori and wild silk fibroin, were used in the 1700 - - - 700 cm region especially. We had also compared the spectra with glycine, alaninen, polyglycine I, polyglycine II, poly - L - alanine (.) , glycine - alanine co - polymer and its mixture.

OUR RESULT AND CONCLUSION.

Typical spectra obtained from bombyx mori and wild silk fibroin are shown table I, respectively.

It will be seen that wild silk fibroin spectra are in good agreement with comparable spectra obtained by - poly - L - alanine.

Although our spectra show several new details in the regions of special interest here.

In particular, it is seen that bombyx mori shows its glycine - glycine skelt. Stretching vibration at 1015 cm just like polyglycine, but wild silk fibroins do not show this position.

Furthermore, we find that wild silk fibroin have C - CH₃(?) vibration at 1308 cm just like . - poly - L - alanine and alanine.

We also suggested that wild silk fibroin film may be changed its configu - ration when the film dropped into alcohol and water for a while and dried

TABLE I.

POLY - L - ALANINE	BOMBYX MORI 1	BOMBYX MORI 2	BOMBYX WILD SILK 1	WILD SILK 2
1305 (. .)	-	-	1305	1305
- (.)	1200	-	1200	-
1108 (. .)	-	-	1108	1108
-	1065	1065	-	-
1050 (. .)	-	-	1050	1050
-	1015	1015	-	-
966 (.)	-	-	-	966
924 (.)	-	-	-	924
908 (. .)	-	-	908	908
893 (. .)	-	-	893	893
	700	-	-	-

1 = film from TFA.

2 = film from alcohol treatment.

out at room temperature.

As a result, that film's spectra show a strong band at 966 cm like in - poly - L - alanine.

So, we suggested that wild silk fibroin which were obtained from TFA solution are not exactly - helix configuration, because - poly - L - alanine do not changed into - poly - L - alanine so easily like wild silk fibroin.

It shows us that wild silk fibroin differ from - poly - L - alanine configuration and something like mixed configuration or amorphous which were obtained from TFA solution.

It may be concluded that it is very difficult to make - helix, because silk fibroin have much long side chains than glycine and alanine.

Anyway, that is why we believed that fibroin have hydrogen bonding in intra

of polypeptide chain like α - proteins, but when it rolled and treatment of alcohol, its intra hydrogen bonding inverted into inter molecular bonding depend on its rotation. However, distinction of two fibroins are significant to the principles of fibroins structure how dominant is the effect of the glycine and alanine.

We have accordingly collected information on the infrared of film of the two different silk fibroins and have some fundamental conclusion.

It will be true that *bombyx mori*, accordingly to the component of silk fibroin, has a correspondingly high proportion of bulky side chains than is found in wild silk fibroin, so tyrosine and other long chain amino acid will be much make amorphous than will silk, because they need large spaces than glycine, alanine and sericine.

The bonded order of silk fibroin have G - A, G - X, A - X and adding to G - G, A - A segments, because we found the characteristic band at 1015 cm, which is ascribed to glycine - glycine skelt. stret. vib. and also we found new bands which are not belong to polyglycine and poly - L - alanine.

Accordingly to the infrared spectra, it is very easy to find the distinction between *bombyx mori* and wild silk fibroin, (table*1) .

As it will be seen that in silk fibroin, however, the 1660 cm band is certainly not always associated with the α - helix form like in proteins. For instance, in collagen, where are no α - helix, the carbonyl bands has this number.

But it is true that in the film which are cast from TFA solution appear to be in a something like folded form, because 1630 cm band is absent, which is belong to the extended form.

1050 cm and 1167 cm bands in wild silk fibroins are ascribed to methyl group, it is therefore very likely that those bands are associated with the C - CH₃ group and may be caused essentially by movement of the methyl groups as a whole, with bending or stretching of the C - C bond.

924 cm and 966 cm band are evenly skeltal modes, since they appear in β - poly - L - alanine but are not in the spectra of the α - form.

Now, we have to decide that the silk fibroin which were made from 1% TFA solution are similar to α - helix type or not, first.

- 1 As silk fibroin configuration are similar to α - poly - L - alanine configuration by infrared spectrum, but fibroin have much different amino acid, and it is very easily to hydrosis, so it is very difficult to consider that those configuration are almost same that of poly - L - alanine.
- 2 It will be seen that amide I band at 1660cm of two silk fibroins are exactly in β - type, but when it comes to treat of alcohol, many bands in 1500 – 700cm region have much changed easily.
As α - poly - L - alanine do not such structural changed, moreover, amide I band is not corresponding with other shifts which are assigned β - type, because amide I band still at 1660cm position.
- 3 We do not believed β - type in silk fibroin, because glycine - glycine structure did not have β - type and also silk fibroin have much different amino acid radicals.
That is why we do not believed that the silk fibroin which were made from TFA solution is something other configuration than β - type.

Is it changed into α - type configuration, after the film into methanol and dried out ?

The film show many absorption in low frequency are exactly same position of α - poly - L - alanine, but amide I band is not perfectly shifted to low frequency like 100% of α - poly - L - alanine and it shows that amide I band in wild silk fibroin is split into two components, that is, 1660cm and 1630cm.

But those relative intensity are very changeable.

On the other hand, bombyx mori has much shifted than those of wild silk and 1660cm band seems to be shoulder of 1630cm absorption, but bombyx mori do not show any β - type band in the low frequency.

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So we consider that the structure of bombyx mori silk fibroin have much complicated hydrogen bonding and chain steric effect than that of wild fibroin.

This means depending to glycine and alanine segments which is less than wild silk fibroin.

On the other hand, wild silk fibroin have much alanine, so some parts are very easy to changed into the typical β -type.

It allows considerable molecular flexibility by rotation around the α -carbon single bonds, because hydrogen bonding in silk fibroin interacted with methanol and water easily than polyglycine and poly-L-alanine.

The difference between two solvents, that is TFA and DCA are perhaps connected with the CH group in DCA, which will have appreciable hydrogen bonding capacity and also OH group in alcohol interacted with hydrogen bonding and chain rotation.