

Near infrared studies on molecular conformation of silk fibroin

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Introduction

Spectroscopy in the near infrared (NIR) region is an invaluable technique for non-destructive analyses of a wide range of samples. In particular, reflectance NIR measurements have been utilized as a probe for the quality control in food and industrial science. Nevertheless, it is also possible to create a practical application of NIR for molecular structure analysis because the NIR region contains overtone and combination bands of normal vibrational mode which are potentially rich in information about molecular conformations.

Silk fibroin is a kind of fibrous protein which has several stable conformations. A few studies have indicated that the structural changes in fibroin cast film was induced by treatment in methanol/water solution. In order to utilize extensively the NIR technique for the determination of protein structure, it is important to establish the correlation between NIR spectra and protein secondary structures. In this study NIR measurements have been carried out to investigate the structural information of silk fibroin.

Experimental

Silk fibroin solutions were directly extracted from the posterior silk gland of mature silkworm (*Bombyx mori* and *Samia cynthia ricini*) larvae and fibroin films were prepared by casting these silk fibroin solutions. Their IR spectra have also been obtained to estimate the secondary structures from the amide I bands (predominantly C=O stretching mode) with band curve-fitting procedures. NIR spectra were measured with a Shimadzu UV-3100S dispersive spectrophotometer. IR spectra were recorded on a Digilab FTS-60A/896 Fourier-transform infrared spectrophotometer at 2 cm⁻¹ resolution.

Results and discussion

The lower wavelength band at 1939 nm in the NIR spectrum (1800–2450 nm) of *Bombyx mori* silk fibroin cast film (Figure 1) is due to the water absorption and five distinct bands are assignable

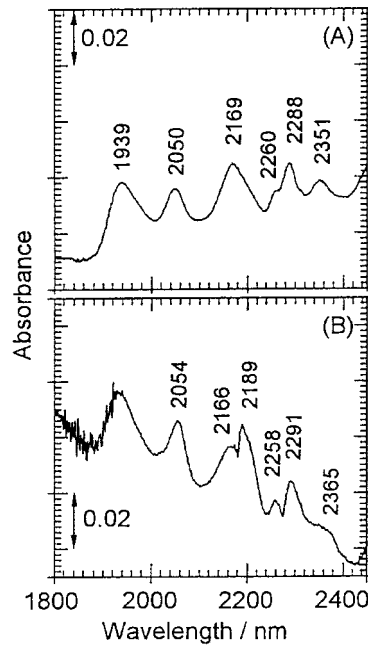


Figure 1. NIR spectra of *Bombyx mori* (A) native and (B) treated fibroin cast films.

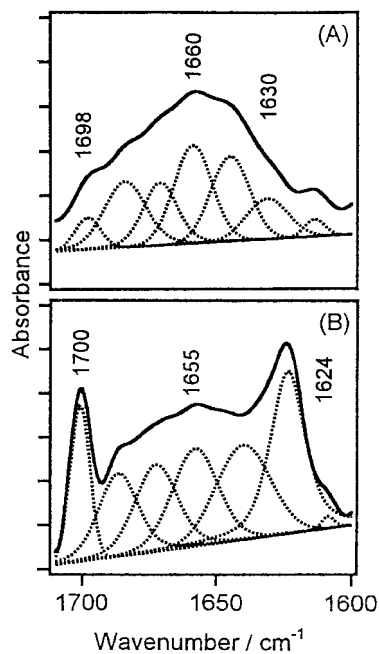


Figure 2. IR spectra of *Bombyx mori* (A) native and (B) treated fibroin cast films.

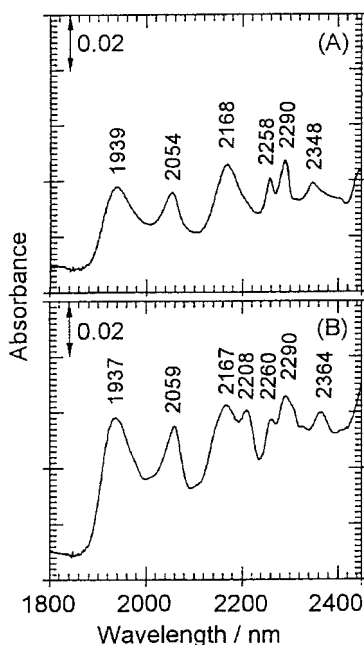


Figure 3. NIR spectra of *Samia cyynthia ricini* (A) native and (B) treated fibroin cast films.

to the protein absorption at 2050, 2169, 2260, 2288 and 2351 nm in native fibroin film. In Figure 2 are exhibited their corresponding deconvolved IR spectra in the amide I region. The amide I bands have been frequently used for analyses of secondary structures of proteins and polypeptides. The bands at 1655 and 1660 cm^{-1} are assigned to the α -helical form and the feature at 1624 cm^{-1} , 1630 cm^{-1} and 1700 cm^{-1} are due to β -sheet structures. The remaining components can be assigned to the unordered and turn structures. These data exhibit that the α -helix was predominant in native fibroin and the β -sheet components increased with the diminution of α -helical form in the methanol/water treated fibroin. Several remarkable spectral changes were also observed in the NIR region. The relative intensity near 2169 nm diminishes with the appearance of a new band at 2189 nm. Previous study suggests that the absorption band at 2170 nm was influenced by the structural changes of proteins.¹ These observations indicated that the band at 2170 nm was ascribed to α -helical form and the one at 2189 nm was related to the β -sheet structure. Figures 3 and 4 show the NIR and IR (amide I) spectra of *Samia cyynthia ricini* silk fibroin cast films. Although the components of amino acids in fibroin are different from those in *Bombyx mori* fibroin, similar α to β structural changes are obtained in amide I region. The NIR feature shows a characteristic new band at 2208 nm which is due to the β -sheet structures as well as decreases in the relative intensity near 2168 nm. Therefore, the feature near 2170 nm can be used for monitoring the transition of secondary structures of several proteins.

The treated fibroin films exhibit a peak at 2350 nm at a much longer wavelength than both of the two fibroins. These high-wavelength shifts near 2350 nm seem also to be characteristic of the structural changes in fibroin films. On the other hand, the band at 2050 nm which is due to amide A/II mode, shows a characteristic upward shift with α to β structural transition. Liu *et al.* noted that amide A/II was sensitive to the destruction of hydrogen bonds in the amides by the thermal

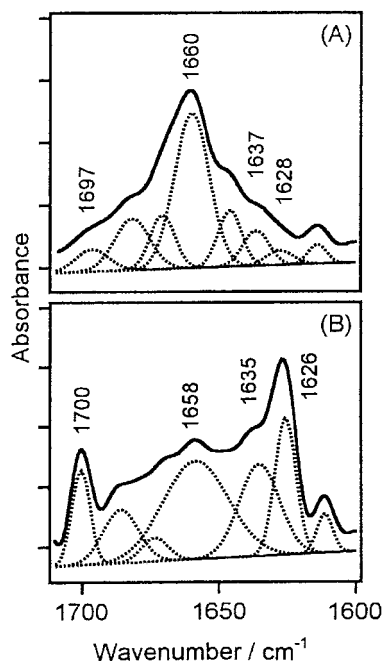


Figure 4. IR spectra of *Samia cynthia ricini* (A) native and (B) treated fibroin cast films.

denaturation.² These observations indicated that amide A/II can also be expressed for intensity of hydrogen bonds for differential secondary structures. Additionally, in order to identify qualitative features of silk fibroin, hydrogen–deuterium exchange experiments have also been carried out.

In summary, we have carried out NIR measurements of silk fibroin which suggest that NIR spectra were sensitive to the secondary structure changes of protein or polypeptides.

References

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2. Y. Liu, R. Cho, K. Sakurai, T. Miura and Y. Ozaki, *Appl. Spectrosc.* **48**, 1249 (1994).