

FTIR Study on Structural Transformation of Keratin Films Induced by Stretching

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Synopsis

Fourier transform infrared spectroscopy (FTIR) was employed to explore the structural transformation by stretching of the film composed of a keratin derivative. The derivative was prepared by *S*-carboxymethylation of the low-sulfur keratin of wool. The cast film was stretched to different ratios in ethanol/water (7:3 v/v). The amide infrared absorption bands of the derivative were changed by stretching the film. Curve analysis was undertaken to estimate the contribution of each peptide conformation to the amide II band at each stretching. The analysis shows that the stretching mainly causes the structural transformation from random coil to β -structure. Moreover, a transient state arises in the course of the transformation. In such a state, the part of random coil increases while that of β -structure decreases. The infrared dichroism of the film also supports the existence of the transient state. On curve analysis, only the α -structure was found to reverse the dichroism of the amide II band in the transient state. This result suggests that the crystalline phase of α -structure is rearranged. Such rearrangement results in inhibition of the transformation from random coil to β -structure.

INTRODUCTION

Keratin derivatives isolated from wool have drawn interest as raw materials for films that exhibit excellent biocompatibility¹ and high sorption capability for metal and organic ions.^{2,3} Several researchers^{1,4,5} have shown that these characteristics are influenced by the conformational states of keratin molecules such as α -form, β -form, and random coil. The keratin derivatives exhibit the conformational changes according to the casting, aftercuring, and stretching of the film. Those procedures have an important role in the application of keratin protein.

The structural transformation caused by stretching has been particularly interesting in connection with the α - β transformation by stretching natural keratin fibers such as wool and horse hair keratin. The results from X-ray diffraction,⁶⁻⁸ infrared spectroscopy,⁹ and differential scanning calorimetry (DSC)¹⁰ show that the α -structure disappears completely in the ultimate steam-stretched keratin protein. *S*-carboxymethylated low-sulfur keratin (SCMKA), however, retains part of the α -structure even in film with 200% stretching either under steam or in ethanol/water, as judged by X-ray

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diffraction.¹¹ This result leads us to predict a transformation process of keratin derivatives alternative to the α - β transformation observed in the natural keratin fibers.

The present study is undertaken to explore the structural transformation in the stretching of SCMKA film by Fourier transform infrared spectroscopy (FTIR). The infrared absorption associated with amide groups of proteins and polypeptides is closely correlated with the conformation of these biopolymers.^{12,13} One may evaluate the conformational state from observed infrared amide bands using the established correlation. In most proteins, including keratin, unfortunately, the amide bands due to each type of conformation overlap too extensively to glean detailed conformational information. One method of solving such a spectral problem is through the use of curve analysis such as band separation and band fitting. Then, the resolution and the frequency precision of observed spectra are an important aspect of the spectral analysis. FTIR is superior to dispersive infrared spectroscopy, which has been employed in studying the conformation. We attempt to apply the curve analysis to the amide bands of SCMKA measured by FTIR with the aid of the established correlation. This procedure enables us to evaluate the structural transformation induced by stretching SCMKA film.

EXPERIMENTAL

Materials

S-carboxymethylated keratin (SCMK) was obtained from Merino 64's wool according to the procedure of O'Donnell and Thompson.¹⁴ The SCMK consists of two different components: One is SCMKA, made up of the low-sulfur keratin in the microfibril. The other, SCMKB, is made up of the high-sulfur keratin in the matrix. The separation was performed by isoelectric precipitation to obtain SCMKA.

The SCMKA film was prepared with 5 mL of 1 w/w% SCMKA formic acid solution on a cylindrical cavity with 5 cm diameter made of teflon block. The film was uniaxially stretched to a desired ratio by a hand-stretching apparatus in ethanol/water (7 : 3 v/v), and air-dried under setting of the apparatus. The samples for FTIR measurement were stocked in a desiccator.

Measurements

Infrared spectra were measured by a Nicolet 5DX Fourier transform infrared spectrophotometer under dried air. The instrument was the single-beam-type equipped with a room-temperature triglycine sulfate detector. The center focus gave an 8 mm diameter image size. Interferograms of sample and background were averaged with 200–400 scans to achieve a better signal-to-noise ratio. A Fourier transform process was performed with apodization of Happ-Gencel function to obtain spectra at a resolution of 4 cm^{-1} . The frequency was calibrated to a 0.01 cm^{-1} accuracy with a helium-neon laser. A Specac wire grid polarizer consisting of 0.2 μm wide strips of aluminum on a KRS-5 substrate was used to obtain plane-polarized infrared radiation. The polarizability, P , of the polarizer was determined to be 97.3% at 2000 cm^{-1} , 97.8% at 1500 cm^{-1} , and 98.1% at 1000 cm^{-1} by transmission measurement

with several polarizers.¹⁵ The imperfection, t , at each wavenumber calculated from P was used to correct the dichroic ratios as described elsewhere.¹⁶

In the dichroic measurement, the stretching axis of the film and the direction of the electric vector of the polarized radiation were always placed at 45° with respect to the vertical direction. This setting enabled us to eliminate the error in the observed dichroic ratio due to the polarization of the beamsplitter in the interferometer of the FTIR spectrometer as well as the prism and grating monochrometers of dispersive infrared spectrometers.¹⁷

The curve analysis was carried out by the curve analysis program (CAP) released from Nicolet Instrument Co. This program, which is executed on the data processor of the FTIR instrument, operates with up to nine synthetic peaks and employs an optimization procedure of the curves using Gauss-Newton algorithm for best fit to an observed band. CAP allows us to interactively specify the parameters of position, width, intensity, and composition as the percent Gaussian versus Lorentzian contribution to each individual curve and to obtain the sum of the curves or the difference curve as a guide toward the optimization of the curves.

RESULTS AND DISCUSSION

Infrared Spectral Changes in Stretch of SCMKA

The SCMKA films with different stretching ratios were measured by transmission FTIR spectroscopy under dried air. Every spectrum is given in absorbance mode as shown in Figure 1; the difference between the maximum and minimum absorbances of each spectrum is designated as ΔA and shown in the figure caption. These spectra have the amide bands that exhibit the

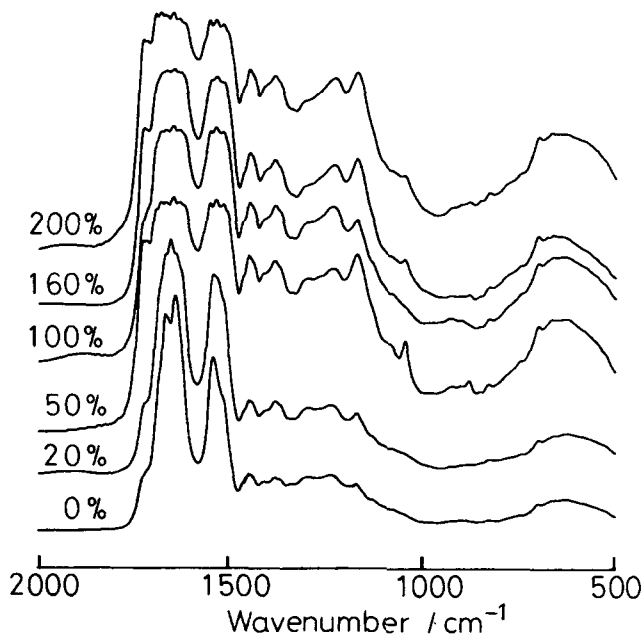


Fig. 1. FTIR spectra of SCMKA film stretched to various ratios: the corresponding values of ΔA are 0.580, 0.636, 0.874, 0.557, 2.689, 3.320 from top.

TABLE I
Frequencies of Amide II used in Curve Analysis^a

Conformation		Frequency (cm^{-1})
Random coil		1535
α -Helix	//	1516
	\perp	1546
Parallel-chain pleated sheet	//	1530
	\perp	1550
Antiparallel-chain pleated sheet	//	1530

^aFrom ref. 13.

characteristics found in protein spectra. In addition, the modification with iodoacetic acid gives a shoulder peak on the amide I band due to the carboxylic acid.

In the amide I and II band ranges, the original film gives main peaks at 1650 and 1546 cm^{-1} and also shoulder peaks at 1685 and 1530 cm^{-1} . The amide I and II bands have been investigated for polypeptide conformations.^{13,18,19} Miyazawa and Blout¹³ have previously shown the correlation of the amide I and II frequencies with the conformations such as random coil, α -helix, antiparallel and parallel β -sheets from the observed and calculated frequencies. As a result, in the present investigation, the original film cast from formic acid solution includes α -structure as well as β -structure, as also pointed out from the results by X-ray diffraction.¹¹

The stretching of the film changes the heights and shapes of the peaks as shown in Figure 1. In particular, the amide bands appear to make the peaks tops ambiguous and the band widths are broadened as the film is stretched. These changes may reflect the α - β transformation by stretching SCMKA film. However, high stretching makes the peak tops so ambiguous that it is

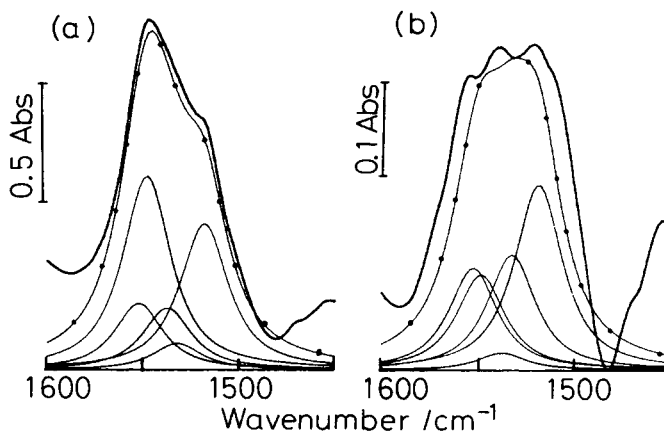


Fig. 2. Curve analysis for amide II bands of SCMKA films stretched to 10% (a) and 100% (b): (—) experimental peaks, (---) individual calculated peaks corresponding to the conformations (see text and Table I), (-·-) overall calculated peaks obtained by taking the sum of their intensities.

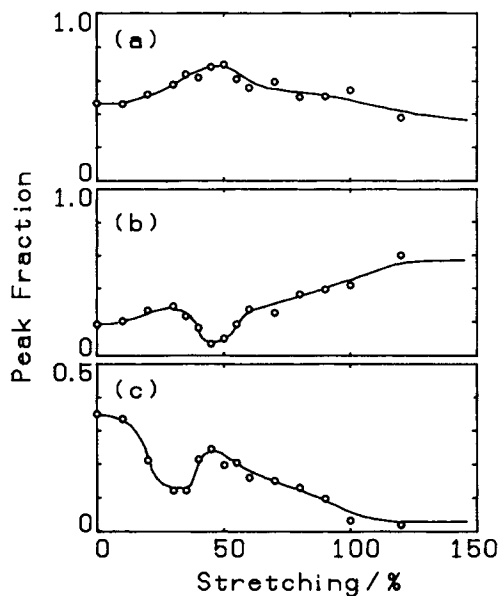


Fig. 3. Peak fractions corresponding to the conformations in stretched SCMK films: (a) α -structure, (b) β -structure, (c) random coil.

difficult to determine the conformational components from the frequencies of the amide I and II bands.

We have tried to analyze the amide II through the curve analysis to evaluate conformational contents on each stretched film. Each component spectrum in the analysis is generated using the following values: The half width is 35 cm^{-1} ; the peak shape is a mixed form of Gaussian and Lorentzian characters (1 : 9); the peak frequencies are set at values corresponding to the conformations obtained by Miyazawa,¹³ and Blout as tabulated in Table I. Figure 2 shows the original spectra and the individual calculated component spectra in the range of the amide II band in the case of the stretching of 10% and 100%. The overall calculated spectrum is in fair agreement with the original spectrum at the low stretching, while it is not so well fitted to the original one at the high stretching, especially on both sides of the original amide II band. This discrepancy seems to originate from the deformation of the peak shape and the broadness of the peak width due to the stretching. However, the ratio of each spectral component has been calculated without using the shape and the width as parameters to avoid making the fitting procedure complicated through numerous parameters.

The areas of spectral components due to the corresponding conformational forms are obtained as fractions of the total component area for each stretched film. Figure 3 shows the changes of the fraction for each structure in the stretching. Note the fractions of β -structure that are the sums of the ratios due to the antiparallel and parallel β -structures. Those peaks have the same peak top so that the peaks cannot be separated into each component of β -structure. In very gross terms, the α -structure content remains unaltered over the stretching ratio. This result indicates that the α -structure is relatively stable to stretching. On the other hand, the random coil is gradually transformed to the β -structure. As a result, stretching in ethanol/water (7 : 3

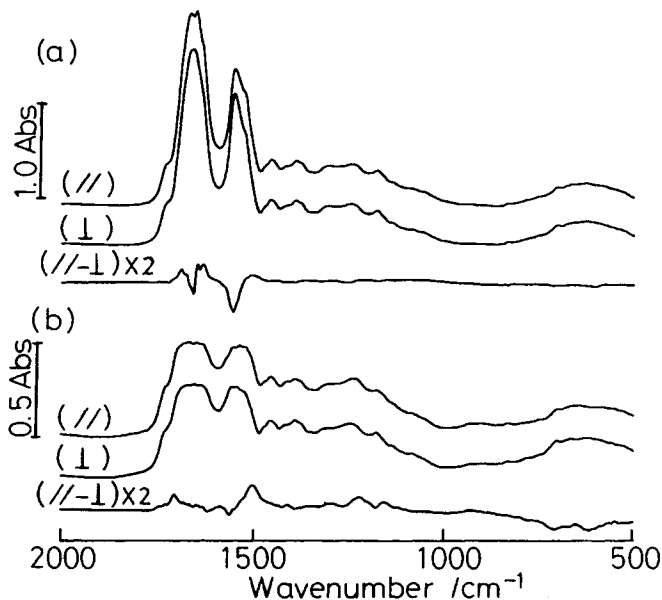


Fig. 4. FTIR spectra of SCMKA films stretched to 10% and 120%: (//) E vector parallel, (\perp) E vector perpendicular to stretching axis, ($// - \perp$) difference spectra of $// - \perp$.

v/v) causes the structural transformation of random coil into β -structure rather than that of α -structure into β -structure.

In connection to these features, it is noteworthy that a transient state appears in the course of the structural transformation near 50% stretching, as shown in Figure 3. In this region, the β -structure content is lowered and the α -structure and random coil contents are raised. This transient state is expected to reflect the functions of SCMKA film. In fact, the permeability of the dye of C.I. Acid Orange 7 rises near 50% stretching, as reported previously.⁵ Such a phenomenon can be ascribed to the increase of the random coil in the transient state.

Infrared Dichroism Changes in Stretching of SCMKA

The infrared dichroism of SCMKA film at different stretching was determined to discuss the structural transformation from the viewpoint of the orientation of the conformations. Figure 4 shows the polarized infrared spectra of the SCMKA films at stretchings of 10% and 120% and the difference spectra between the spectra with E parallel and perpendicular to the direction of the stretching. The difference spectra distinctly exhibit the wavenumber range where the films give infrared dichroism. The amide bands give the most appreciable change of the dichroism in stretching. Figure 5 shows the dichroism difference spectra of the films stretched to different ratios in the amide I and II bands. Those difference spectra reflect the change of orientation for each crystalline structure.

Natural keratin fibers are generally called α -keratin in the native form and β -keratin in the stretched form. This designation comes from the X-ray diffraction patterns⁶⁻⁸ and the infrared dichroic spectra.⁹ Taking into account that in low stretching, the SCMKA film contains mainly α -structure and in

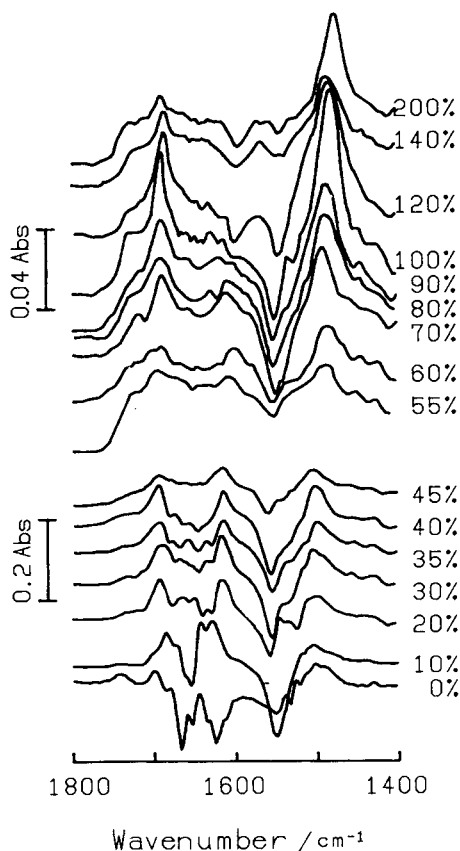


Fig. 5. Variations of difference spectra with stretching of SCMKA film.

high stretching, contains substantial β -structure, the dichroic frequencies for both keratin forms can be estimated in amide I and II bands as tabulated in Table II. The table also includes the corresponding frequencies for horse hair keratin.⁶ Comparison of the data in Table II shows that the frequencies of amide I and II for SCMKA are almost identical with those observed with the horse hair keratin. This means that SCMKA forms crystalline structures similar to those formed by natural keratin fibers.

TABLE II
Frequencies of Amide I and II Bands Observed by Dichroism Difference Spectra

Infrared band	Form ^b	Keratin ^a (cm^{-1})	SCMKA (cm^{-1})
Amide I	$\alpha(\parallel)$	1655	1650
	$\beta(\parallel)$	1627	1610
	$\beta(\perp)$	1697	1695
Amide II	$\alpha(\perp)$	1545	1560
	$\beta(\parallel)$	1522	1500

^a From ref. 9.

^b These forms are observed under less than 10% of humidity and often denoted by α' and β' .

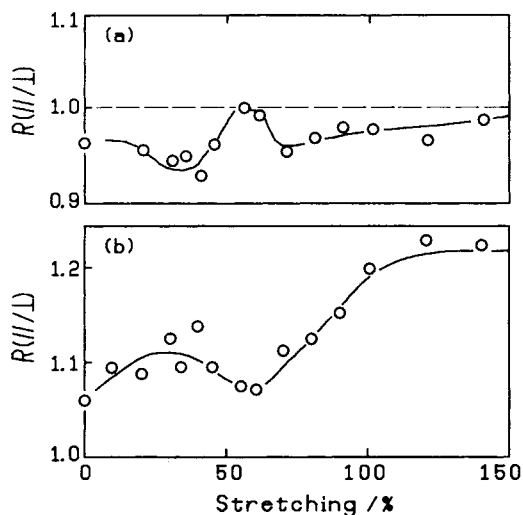


Fig. 6. Changes of infrared dichroic ratios at (a) 1560 cm^{-1} and (b) 1500 cm^{-1} in stretching SCMKA film.

The dichroic ratio was calculated from intensities at 1560 and 1500 cm^{-1} to see how stretching changes the orientation of each conformational form. These frequencies are respectively assigned to the characteristic vibrations for α - and β -keratins in amide II. Figure 6 shows that the perpendicular dichroism at 1560 cm^{-1} due to α -keratin decreases, but the parallel dichroism at 1500 cm^{-1} to β -keratin increases with an increase of stretching, except in the range near 50% stretching. The dichroic change near that stretching ratio also supports the existence of the transient state in the structural transformation. It should be noted, however, that the dichroic ratio obtained in the above procedure is not a correct value for the orientation of each conformational

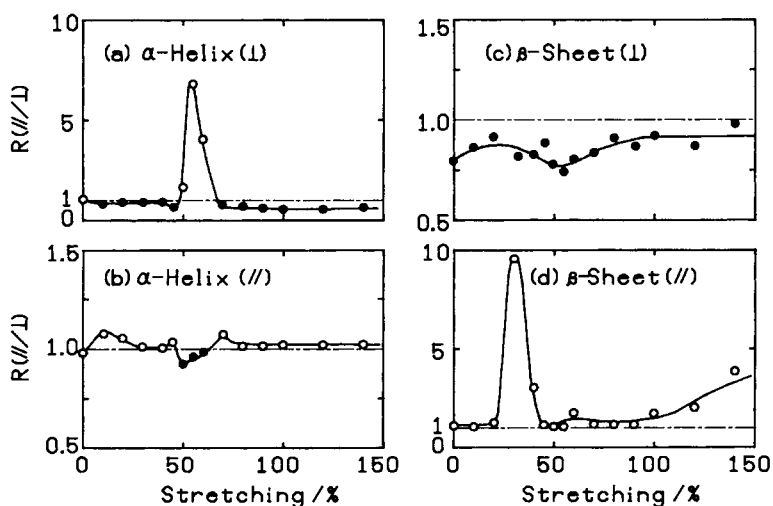


Fig. 7. Infrared dichroic ratios corresponding to the frequency modes of α - and β -structures for extended SCMKA films: (O) parallel and (●) perpendicular dichroism to stretching axis.

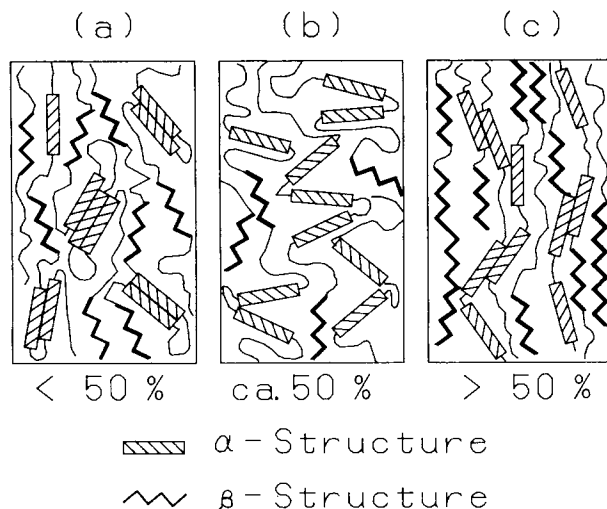


Fig. 8. Schematic illustration of structural transformation and rearrangement with stretching of SCMKA film.

structure. It is only a parameter revealing the trend of dichroism at a noted frequency in stretching. The intensity at the frequency results from the overlap of peaks due to the conformational structures.

The curve analysis for amide II band in the dichroic spectra was undertaken to calculate the correct dichroic ratio for each structure. The values used in this analysis are the same for the transmission infrared spectra as described above. Figure 7 shows the dichroic changes for the components at the frequencies of α - and β -structures. The resulting dichroic ratios show that in all the cases except the orientation of α -structure near 50% stretching, the α - and β -structures are oriented along the stretching axis. The dichroism of α -structure is reversed in this range so that the α -structure is oriented across the stretching axis. Such a reversal of dichroism accompanied by stretching of polymers was also found for high crystalline polyvinylchloride.^{20,21} In relevant reports, it was concluded that high stretching rearranges intramolecular crystallite into intermolecular crystalline form. A similar rearrangement, as shown in Figure 8, may occur in the crystallite of α -structure since the α -structure is stable to stretching, as described above. It is reasonable that such a rearrangement depresses the formation of β -structure and, consequently, leads to an increased random coil. Thus, the process of rearrangement provides a satisfactory explanation for the occurrence of the transient state in the structural transformation.

It is interesting that the α -structure of SCMKA is stable to stretching. Such stability has not been found in any α -structure of synthetic polypeptides. The stability seems to be inherent in keratin proteins, because the α -structure of keratin proteins forms a coiled-coil rope alternative to the helix coil of the polypeptides.²² However, natural keratin fibers such as wool and horse hair yield no stability of α -structure to stretching. In fact, the α -structure of the natural keratin fibers begins to disappear even at low stretching, according to X-ray diffraction⁶ and DSC¹⁰ results. The difference between the keratin fibers and the keratin derivative may be attributed to the cross-links

between peptide chains in the keratin fibers. A detailed study on the effect of disulfide bond on the structural transformation is in progress.

References

1. K. Itoh, T. Miyamoto, H. Inagaki, and Y. Noishiki, *Kobunshi Ronbunshu*, **39**, 249 (1982).
2. M. S. Masie and M. Friedman, *J. Appl. Polym. Sci.*, **18**, 23, 675 (1974).
3. C. L. Bird, *The Theory and Practice of Wool Dyeing*, 4th ed., Chorley & Fichersgill, Leeds, 1972.
4. K. Joko, J. Koga, and N. Kuroki, *Sen-i Gakkaishi*, **39**, 198 (1983).
5. J. Koga, Y. Kawaguchi, K. Joko, N. Ikuta, and T. Hirashima, Proc. Int. Cong. Membranes and Membrane Processes, Tokyo (1987) p. 277.
6. E. G. Bendit, *Textile Res. J.*, **30**, 547 (1960).
7. A. R. B. Skertchly, *J. Textile Inst.*, **51**, T517 (1960).
8. K. Arai and S. Arai, *Int. J. Biol. Macromol.*, **2**, 361 (1981).
9. E. G. Bendit, *Biopolymers*, **4**, 539 (1966).
10. J. Koga, M. Shibano, and E. Nishio, *Chem. Lett.*, **1987**, 265.
11. H. Sakabe, T. Miyamoto, and H. Inagaki, *Sen-i Gakkaishi*, **37**, 273 (1981).
12. T. Miyazawa, T. Shimanouchi, and S. Mizushima, *J. Chem. Phys.*, **29**, 611 (1958).
13. T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).
14. H. O'Donnell and E. P. O. Thompson, *Aust. J. Biol. Sci.*, **17**, 973 (1964).
15. G. Rupprecht, D. M. Ginsberg, and J. K. Leslie, *J. Opt. Soc. Am.*, **52**, 665 (1962).
16. M. Tsuboi, *J. Polym. Sci.*, **59**, 139 (1962).
17. E. Charry, *J. Opt. Soc. Am.*, **45**, 980 (1955).
18. J. L. Koenig and B. Frushour, *Biopolymers*, **11**, 1871 (1972).
19. T. J. Yu, J. L. Lippert, and W. L. Peticolas, *Biopolymers*, **12**, 2161 (1973).
20. M. Tasumi and T. Shimanouchi, *Spectrochim. Acta*, **17**, 731 (1961).
21. M. Ahina and S. Enomoto, *Nippon Kagaku Zasshi*, **81**, 1370 (1960).
22. E. G. Bendit and M. Feuhelman, in *Encyclopedia of Polymer Science and Technology*, Vol. 8, H. Mark and N. G. Gaylord, Eds., Wiley, New York, 1968, pp. 1-14.

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