

Grafting onto Wool. III. Relationship Between Alpha- and Beta-Forms in Wool Keratin of Grafted Fibers

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Synopsis

A quantitative study of the change in the x-ray intensity diffracted from the α - and β -crystalline phases of grafted fibers was carried out. It was demonstrated that a part of the α -helical sections is disrupted by the deposition of polymer and that during extension processes a marked stabilization for the remainder results from the interaction between the residues on the α -helix and the rigid polymer chain. It was also considered that the disrupted sections are identical to the portions which would be easily deformed by stretching. The results on fibers containing hydrophilic polymer suggested little possibility that the β -chain crystal is produced from the disrupted chains of α -crystallites. The mechanism of the so-called α - β transformation was proposed as $\alpha \rightleftharpoons M$, $M^* \rightleftharpoons \beta$.

INTRODUCTION

In the early 1930's, Astbury and his co-workers¹⁻³ found that the x-ray pattern of stretched keratin was quite different from that of unstretched keratin. They reported that little change occurred in the x-ray diagram up to 20%-30% of extension and that marked change appeared at 30%-50% extension. This process was reversible in nature. In the original folded-chain model,⁴ the process was thought of as an unfolding of α -crystalline structure into an extended β -structure, so-called "molecular" α - β transformation: a transition from the regularly folded α -form into stretched β -form from the same chains. It is now universally accepted that the chain conformation in the unstretched state of keratin fibers which gives the characteristic α -pattern is closely related to the α -helix of Pauling, Corey, and Branson.⁵ The model that accounts fully for the simultaneous appearance of the 5.15 and 1.5 Å in meridional arcs was proposed independently by Pauling and Corey⁶ and Crick.⁷ Crick pointed out that the keratins may contain pairs of α -helices distorted so as to twist uniformly around each other, thus producing a two-strand rope and that a three-strand rope is also possible. Pauling and Corey⁸ have also designed a seven-strand cable, six helices twisting about a seventh. Fraser and MacRae⁸ have also presented the idea of a segmented rope with an average

pitch similar to that of a uniformly distorted three-strand rope concept, in which the segments are short, straight sections of α -helices. Lundgren and Ward⁹ considered a more open structure, three-strand aggregates of α -helices with about 70 Å longer straight-segmented sections. On the basis of electron microscopy, Fraser and others^{10,11} suggested the microfibrillar structure of nine protofibrils arranged in a circular ring surrounding a central pair, each of which consists of a three-strand rope of α -helices, with a diameter of 20 Å.

The molecular mechanism involved in the α - β transformation has been a major importance to the determination of the keratin structure. In 1957, Bendit¹² reported that the unfolding of α -helices and the formation of β -crystallites begins at a few per cent of extension and that there is a lack of correspondence between the intensity of the disappearing α -reflection and the appearing β -reflection. He threw some doubt upon the concept of the molecular α - β transformation, and asserted that noncrystalline material might be involved in the production of the β -structure. As far as the transformation of α -helices into the β -pleated sheet structure involving the regular assembly of protofibrils is concerned, stereochemical difficulties arise. Considerable restraints result from entwining and from covalent crosslinking that appear to prevent each other from straightening.¹³⁻¹⁵ At present, this problem is not satisfactorily resolved.

On the basis of the reaction kinetics,¹⁶ and with the knowledge of stress-strain properties,¹⁷ electron microscopy,¹⁸ and chemical stress relaxation of the system,¹⁹ it was proposed that a marked interaction between polymer and wool chains arises which is greatly influenced by the stability of the wool structure. The electron-microscopic evidence showed that the grafted polymer is located between the microfibril and matrix or around the protofibrils. It might be expected that the restraints resulting from an interaction between the side chains of α -helices and rigid polymer are considerable.

The present investigation is an attempt to determine the intensity variation of the three prominent reflections of the wide-angle x-ray diagram of wool in the grafted fibers; and to determine the meridional arc at 5.1 Å and the equatorial spot at 9.8 Å characteristic of α -keratin, and the equatorial spot at 4.65 Å of the extended β -keratin. A quantitative study of the change in the x-ray intensity of the α - and β -reflections involved in the grafted fiber may throw some light on the exact nature of the α - β transformation.

EXPERIMENTAL

Preparation of Grafted Fiber

The tops of fine Australian Merino wool fibers were grafted with methyl methacrylate (MMA) in an aqueous Br^- - $\text{S}_2\text{O}_8^{2-}$ redox reaction system containing diethylene glycol monobutyl ether (BC) as a cosolvent. Homopolymers were not formed in the system. After the reaction period, the

wool fibers were washed only with water and air dried. In studying the diffraction of polymer-wool systems, there are advantages in using the wool-poly(methyl methacrylate) graft copolymer. The advantages are as follows: (1) diffraction halo from the amorphous polymer appears in the vicinity of $2\theta = 14.5^\circ$, which does not overlap on the characteristic reflections of α - and β -keratin, and (2) uniform deposition of polymer in fiber is possible.^{18,20} For the preparation of the grafted fibers in which the fine polymers are uniformly distributed in the wool structure, the following two different reaction systems were chosen for the preparation of grafted wool fibers²⁰: (1) the NH_4Br system (1 g wool, 10 g NH_4Br , 0.3 g $\text{K}_2\text{S}_2\text{O}_8$, 50 g BC, 35 g H_2O , and 5 g MMA, at 18°C ; or 1 g wool, 15 g NH_4Br , 0.3 g $\text{K}_2\text{S}_2\text{O}_8$, 40 g BC, 39.5 g H_2O , and 5 g MMA, at 30°C), and (2) the LiBr system (1 g wool, 27.5 g LiBr, 0.2 g $\text{K}_2\text{S}_2\text{O}_8$, 25 g BC, 44.8 g H_2O , and 5 g MMA, at 18° and 30°C).

Density of Grafted Fiber

The density of MMA-grafted fibers was measured at $20^\circ \pm 1^\circ\text{C}$ by using a density-gradient column consisting of an aqueous solution of potassium iodide.

X-Ray Diffraction

X-Ray diffraction traces were recorded with a Rigaku diffractometer, incorporating a scintillation counter whose output is fed through a pulse-height analyzer to a chart recorder. Nickel-filtered copper radiation was used at 30 kV and 16 mA. The incident beam through a pinhole collimeter had an irradiated area of 3 mm^2 at the specimen, at approximately 18.5 cm from the x-ray source. Considering the small intensity changes brought about by stretching, reproducibility of the counter traces was significant. The constancy of the radiation from this source was periodically checked throughout the work. The x-ray tests were carried out at 65% R.H. and 20°C .

Preparation of Specimen

To evaluate the effects of polymer on the reflections, it was necessary to divide the peak intensity of a reflection into two parts ascribed to the polymer and wool, respectively. Before this operation is carried out, the effects of absorption must be appreciated, since they cannot be ignored for precise treatment of intensity variation which is dependent upon the characteristic reflections under investigation.^{12,15} The amount of absorption which takes place during the transmission of x-radiation through the specimen can be calculated. The absorption depends upon the shape of the specimen and the atomic composition of the mass. A plate in the specimen geometry has the advantage of making it possible to apply the calculation to the upper levels of reflections, such as the meridional reflection at 5.1 \AA .

A fiber bundle was stretched in water at 45°C and dried at room temperature. The bundle was placed with a 15% methanol solution of poly-

(vinyl acetate) in a groove 1.1 mm wide, 15 mm long, and 8 mm deep, hollowed out of a brass plate, compressed in a direction perpendicular to the fiber axis until the pressure had reached 50 kg/cm², and then dried in a vacuum chamber under the applied load. These processes were carried out gradually to avoid disrupting the structure as a whole. In this way, the required specimen (thickness of 1.1 mm) could be obtained. It was found that the poly(vinyl acetate), which acts as a binder, was distributed uniformly in the interstitial regions of the closed packed array of fibers in the specimen, and that the amount of the binder was approximately 30% of the weight of the fibers in each corresponding specimen.

Diffraction Intensity of Wool Fraction

The transmission factor for reflection is defined by eq. (1):

$$T = \frac{\Sigma KI}{\Sigma KI_0} \quad (1)$$

where I and I_0 are the intensity which is diffracted and the intensity which would be diffracted if there were no absorption, respectively; and K is the fraction of the intensity of the incident beam diffracted by the specimen. The x-rays are absorbed by matter and the intensity is reduced by a factor $e^{-\mu t}$ according to the law

$$I = I_0 \exp(-\mu t) \quad (2)$$

where t is the thickness of the specimen and μ is a parameter known as the linear absorption coefficient, which may be given by eq. (3):

$$\mu = \rho \sum_i p_i \mu_{mi} \quad (3)$$

where ρ is the effective density of the system, p_i is the fraction by weight of element, and μ_{mi} denotes the corresponding mass absorption coefficient. The atomic composition of the specimen was estimated from the amino acid composition of Merino wool,²¹ the contents of the grafted polymer and the poly(vinyl acetate), and the moisture regain at 65% R.H.¹⁷

Thus, the quantity $e^{-\mu t}$ must be integrated over the irradiated volume of the matter to obtain eq. (4) for the wool specimen:

$$\begin{aligned} T &= \frac{\int^v KI_0 \exp(-\mu t) dV}{\int^v KI_0 dV} = \frac{1}{V} \int^v \exp(-\mu t) dV \\ &= \frac{\cos 2\theta}{\mu t_0 (1 - \cos 2\theta)} \{ \exp(-\mu t_0) - \exp(-\mu t_0 / \cos 2\theta) \} \quad (4) \end{aligned}$$

where t_0 is the thickness of the plate. The transmission factors for the prepared specimen were computed at Bragg angles of 3–40° according to eq. (4). Some results are shown in Figure 1.

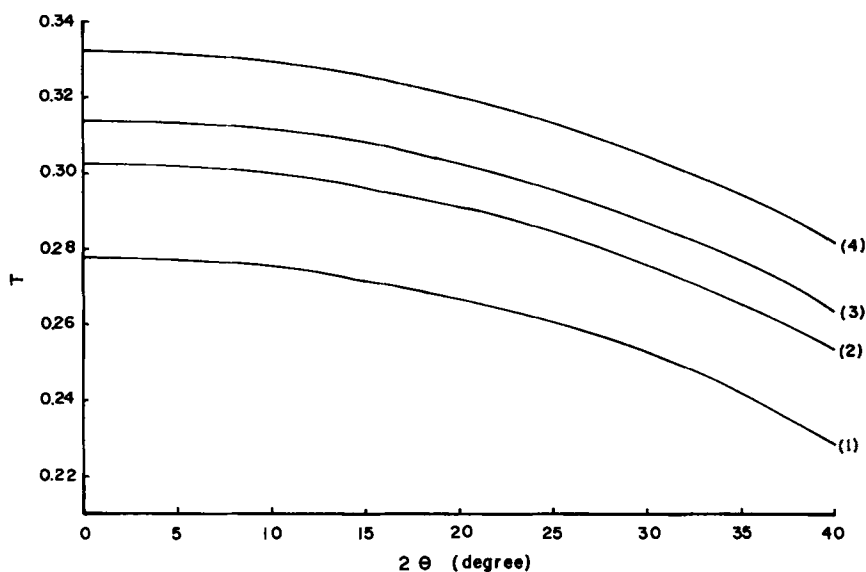


Fig. 1. Transmission factor for various specimens examined: (1) native wool + PVAc ($\mu = 11.26$); (2) 25.8% MMA graft + PVAc ($\mu = 10.49$); (3) 37.5% MMA graft + PVAc ($\mu = 10.07$); and (4) 71.2% MMA graft + PVAc ($\mu = 9.65$).

Assuming no absorption of the x-rays by specimens, the diffraction intensity from wool materials per unit thickness, I_w , is given by eq. (5):

$$I_w = \frac{(i/T) - (A + I_p + B)}{t_w} \quad (5)$$

where i is the observed x-ray intensity at a reflection, and t_w is given by eq. (6):

$$t_w = t_0(100/\rho_w) / \{ (100/\rho_w) + (G/\rho_p) \} \quad (6)$$

where $\rho_w = 1.314$ (from King²²) and $\rho_p = 1.183$; A is the intensity due to air scatter without specimen in the x-ray beam; and I_p and B are the intensities in the absence of absorption, which is attributed to the graft polymer and the binder, respectively. The values of I_p and B can be determined from the determination of the content of polymer and binder in the specimen and of the reflected intensity from homopolymer film with constant thickness.

A similar treatment for the native wool specimen was also carried out by substituting $I_p = 0$ and $G = 0$ in eq. (5). The result, illustrated in Figure 2, demonstrates that there is little deviation from the additive property for the grafted fibers for which the polymer fraction is 0.3 or less. This fact could also be substantiated by the measurements of extent of swelling by the polymer uptake. If the specific volume of each component is additive in this system, the increment in cross-sectional area by the graft polymer is changed by a factor $\rho_w G / 100 \rho_p$. It was found that the average area of

cross section of the native fiber, $380 \mu^2$, was increased by 551 and $530 \mu^2$ for the 37.5% MMA-grafted fibers by the LiBr and NH_4Br systems, respectively, which is very close to the value calculated by the factor, namely $537 \mu^2$. It is clearly suggested that the effective density ρ of each grafted fiber can be estimated from the densities of the components even though interaction of polymer to wool chains occurs. Thus, the x-radiation system required for eq. (5) can be attained for the grafted fibers in which polymer is present to 40% or less at the levels of grafting. Assuming that the fibers are hexagonally close-packed in the specimen, the irradiated volume of the fibers is always constant and independent of the variations in diameter of the fibers which have been stretched to different levels. Through the experiments, I_w was directly traced in the range $2\theta = 4^\circ$ to 40° . There was no need of the correction factor to normalize the peak intensity at the reflections, since the intensities obtained at various extensions were very similar at the high Bragg angles (35° – 40°).

For determining the degree of orientation of the α -crystallites in the grafted fibers, the azimuthal intensities of the reflection in the vicinity of $2\theta = 9^\circ$ were measured by automatically rotating the specimen vertical to the x-ray beams. The degree of orientation, π , was calculated by eq. (7):

$$\pi = [(180^\circ - H^\circ) \times 100]/180^\circ \quad (7)$$

where H° is the half-width value.

RESULTS AND DISCUSSION

Density of Grafted Fibers

If the wool and the grafted polymer are separated into two phases in the fiber with no disruption of the α -crystallites, the masses of the two parts are additive. Thus, eqs. (8) and (9) will be obtained for 1 g of the fiber:

$$1/\rho = (1 - x)/\rho_w + x/\rho_p \quad (8)$$

or

$$1/\rho = 1/\rho_w + \left(\frac{\rho_w - \rho_p}{\rho_w \rho_p} \right) x \quad (9)$$

where ρ , ρ_w , and ρ_p are the densities of the total fiber, wool, and isolated graft polymer, respectively. The polymer fraction x is given by $G/(G + 100)$, where G is the per cent extent of grafting. The plots of $1/\rho$ versus x in Figure 2 illustrate a marked deviation from the ideal line calculated from the equation, suggesting that the polymer phase is not entirely separated from keratin material. The deviation appears to be somewhat greater in a series of the fibers grafted by the LiBr system than for a similar series grafted by the NH_4Br system. This may be due to a finer dispersion of polymer resulting from the greater ability for fiber swelling by the LiBr reaction system, which appears to be compatible with the result obtained from the calculation of the average space occupied by single polymer chains.¹⁹

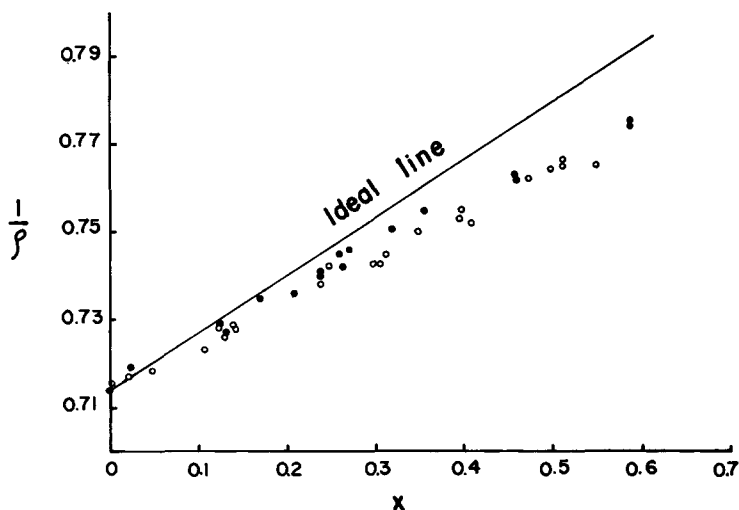


Fig. 2. Relationship of $1/\rho$ vs. x for grafted samples obtained by Br^- - $\text{S}_2\text{O}_8^{2-}$ systems under various grafting conditions: (O) $\text{LiBr-K}_2\text{S}_2\text{O}_8$; (●) $\text{NH}_4\text{Br-K}_2\text{S}_2\text{O}_8$.

The reason for the density of the sample being higher than the density obtained from calculations may be that impermeation of water molecules into the regions among the interacted chains resulted from a decrease in the excluded volume of the copolymer chains which is enhanced by the increase in the content of the hydrophobic polymer.

Equatorial and Meridional Intensity Variation During Extension

Typical results for the equatorial scattering intensity I_w of the native and the grafted wool fibers at various extensions are shown in Figure 3. These observations for the native wool fiber agree with those of Bendit¹² and Skertchly¹⁵; the α -peak height at 9.8 \AA decreases with increase in extension, and the β -reflection at 4.65 \AA increases simultaneously. For the grafted fiber with 37.5% MMA initiated at 30°C by the LiBr system, approximately similar behavior in variation of the peak intensities during the α - β transformation is found regardless of the nature of polymer deposited.

On the other hand, for the 37.5% MMA-grafted fiber initiated at 30°C by the NH_4Br system, a striking difference is observed in behavior of the reflection in the vicinity of $2\theta = 9^\circ$. There is a small change in intensity at the characteristic α -reflection, but the intensity at the 4.65 \AA β -reflection is always increased considerably. It should be noted that the peak position of the α -reflection is shifted by $2\theta = 9.6^\circ$ even at 0% extension. Similar shifts in the peak were also observed for the native wool fiber at high levels of extension.^{12, 15}

The fine structure of the equatorial α -reflection consists of maxima at about 12.2, 11.2, 10.4, 9.2, and 7.8 \AA .²³ It has been reported that the

positions of maxima are closely related to the lateral dimensions of microfibril which depend in turn on the arrangement of protofibrils.²⁴ It has also been proposed that the deposited polymer greatly alters the arrangement of protofibrils in crystalline microfibril, as indicated by electron microscopy, and that the actual location of graft polymer is profoundly dependent upon the conditions used for the graft copolymerization. Compared with the LiBr system, the NH_4Br system brings about the most marked changes in

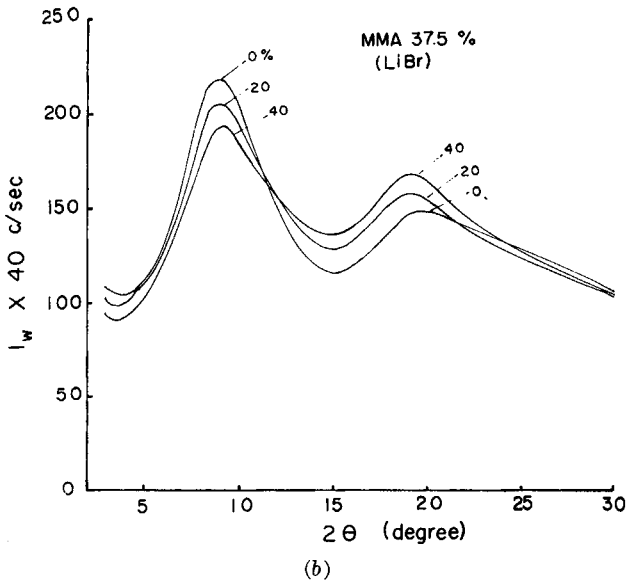
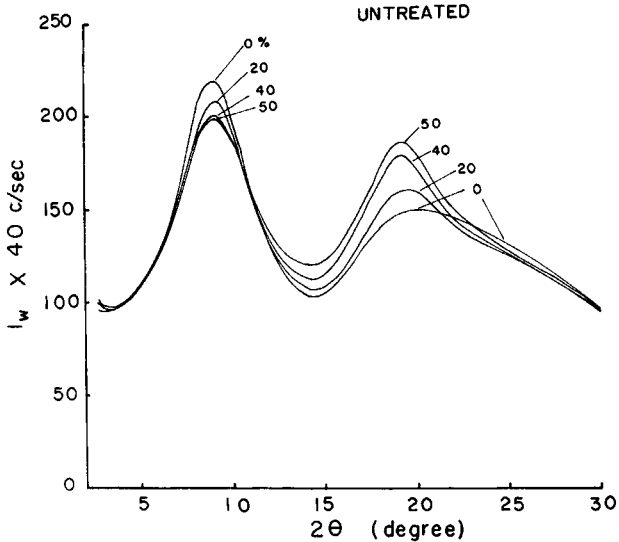


Fig. 3 (continued)

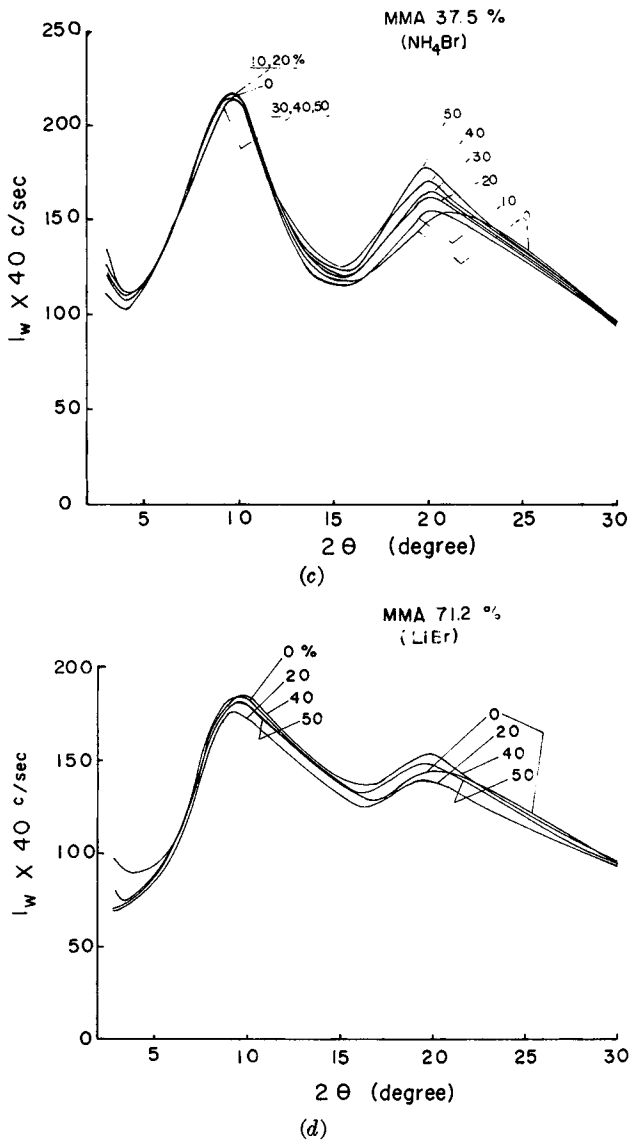


Fig. 3. Variation in equatorial scattering intensity I_w at various extensions: (a) native wool; (b) 37.5% MMA graft (LiBr); (c) 37.5% MMA graft (NH₄Br); (d) 71.2% MMA graft (LiBr).

the salient features of the x-ray diffraction patterns, especially in the region of the crystallographic α - β transformation. However, over the levels of grafting at about 70%, no observable intensity change was recognized during extension, as shown in Figure 3d. A considerable decrease in the α -peak height occurs at no extension. It is worth noting, however, that no appreciable changes in the degree of orientation are produced even at the highest levels of grafting, as shown in Table I.

TABLE I
Degree of Orientation of the α -Crystallites in Various Grafted Fibers with Methyl Methacrylate

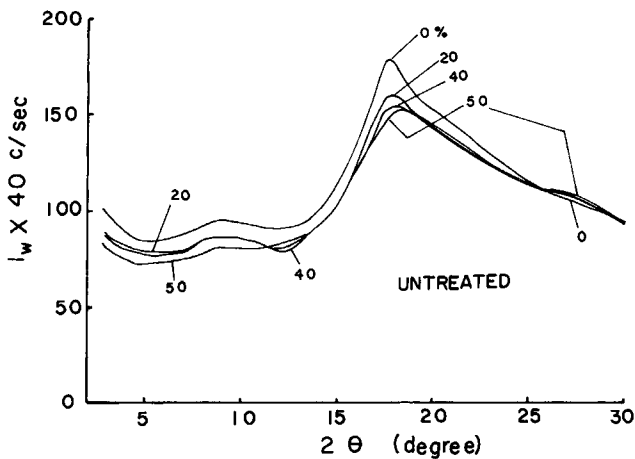
Sample	π , %
Native wool	76.7
Graft, 25.8% ^a	74.2
Graft, 37.5% ^a	73.9
Graft, 37.5% ^b	74.2
Graft, 71.2% ^a	72.3

^a LiBr system, 30°C.

^b NH₄Br system, 30°C.

As can be seen in the x-ray diagrams of the native and the MMA-grafted fibers prepared by the lesser-swelling system of aqueous NH₄Br, the 9.8 Å reflection might be represented by a Gaussian distribution function. Judging by the appearance of the I_w -versus- 2θ curves for the fibers with over 28.5% of polymer grafted by the swelling system, it is likely that a new broad reflection occurs in the vicinity of $2\theta = 14^\circ$, as shown in Figure 3b. The broad reflection rises remarkably with increase in extension and also with increase of the extent of grafting. It is interesting to note that the reflection at $2\theta = 14^\circ$ appears at no extension for the 71.2% MMA-grafted fibers.

It has been found that grafted polyacrylonitrile chains are oriented along the direction of the fiber axis for both silk fibroin²⁵ and rayon fibers.²⁶ This evidence could suggest that such a phenomenon as radicals growing preferentially along the fiber axis direction could also be expected for the case of the polymerization of amorphous poly(methyl methacrylate), especially in the structure with well-oriented wool chains disaggregated by the action of LiBr; and, as a result, a finer dispersion of polymer chains would occur.²⁷



(a)

Fig. 4 (continued)

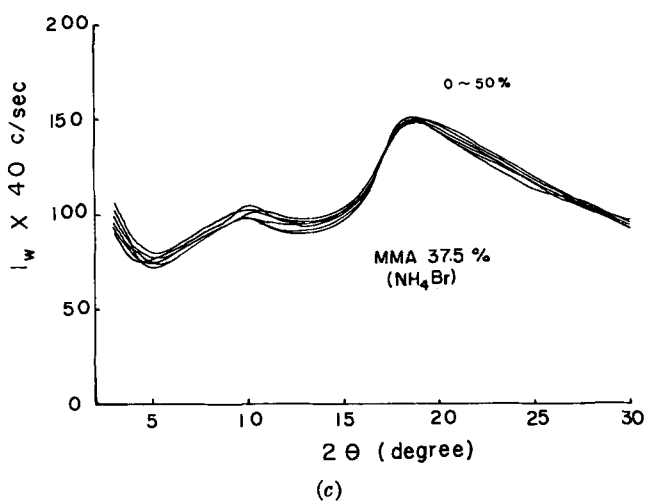
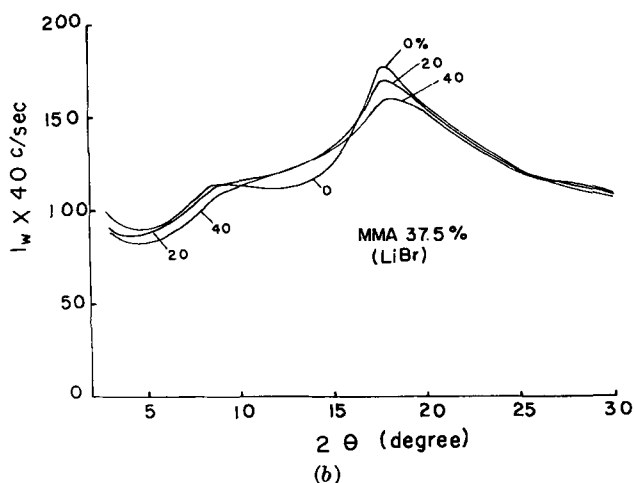


Fig. 4. Variation in meridional scattering intensity I_w at various extension: (a) native wool; (b) 37.5% MMA graft (LiBr); (c) 37.5% MMA graft (NH_4Br).

On the other hand, the polymer applied in the NH_4Br system may tend to be localized within a region between the aggregates such as a microfibril and a matrix, and this leads to a marked stabilization of the α -crystallites in extension.

The curves in Figure 4 show the meridional scattering intensities of the native and the grafted fibers at various extensions. There is a general agreement between the intensity variations for the 5.1 \AA α -reflections and for the equatorial α -reflections at 9.8 \AA for each system. The sharp maxima of the 5.1 \AA reflection peak for either the native or the grafted fiber prepared by the LiBr system tend to disappear with increase in extension and to shift to higher Bragg angles. On the other hand, the following very different features can be seen in the diffraction curves of the grafted fiber

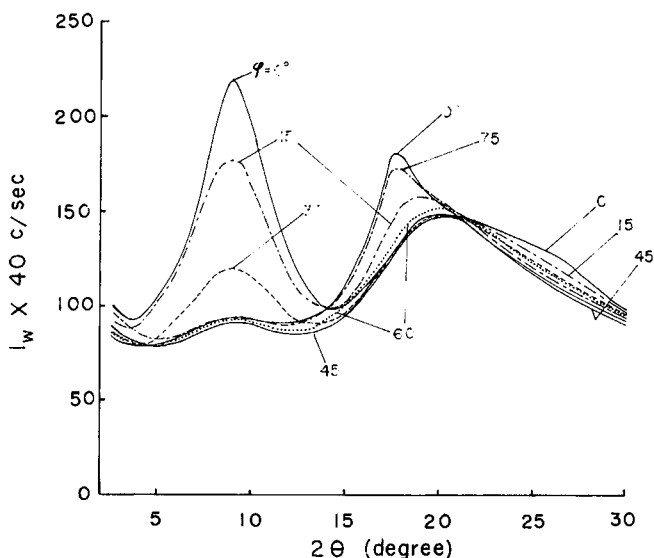


Fig. 5. Variation in scattering intensity I_w at various azimuthal angles for native fibers at zero extension.

prepared by the NH_4Br system (in spite of the fact that no substantial change in longitudinal dimensions was produced by the grafting)¹⁷: (1) the sharpness at the 5.1 \AA reflection is lost at zero extension; (2) the peak position is shifted by $2\theta = 18.5^\circ$, which is approximately the same level as the total shift for the native fiber at 50% extension; and (3) the peak intensity at the initial state is retained even at the higher extension. It is proposed, therefore, that the α -crystallites are partly disrupted by the lateral swelling resulted from polymer deposition, but that the remainder of the material, however, is stabilized by the interactions between the polymer and the side-chain groups of residues forming the α -helices.

X-Ray Intensity Diffracted from the Crystalline Phase of α -Keratin

Figure 5 shows the scattering intensities from the native wool fiber at zero extension at various azimuthal angles ($\varphi = 0^\circ, 15^\circ, 30^\circ, 45^\circ, 60^\circ, 75^\circ$, and 90°). Assuming that the diffracted intensity from the amorphous phase in the oriented crystalline materials is the minimum intensity of the azimuthal reflection at a given spacing, the degree of crystallinity can be estimated at 9.8 \AA reflection by using eq. (10):

$$\frac{(I_{\text{tot}} - I_{\text{amor}})}{I_{\text{tot}}} \times 100 (\%) \quad (10)$$

where I_{tot} is the total intensity at $\varphi = 0^\circ$, and I_{amor} is the intensity at $\varphi = 45^\circ$. At extended states, I_{amor} is the intensity at $\varphi = 90^\circ$ as illustrated in Figure 6. The degree of crystallinity was estimated to be 59.0% at zero extension and 54.5% at 50% extension.

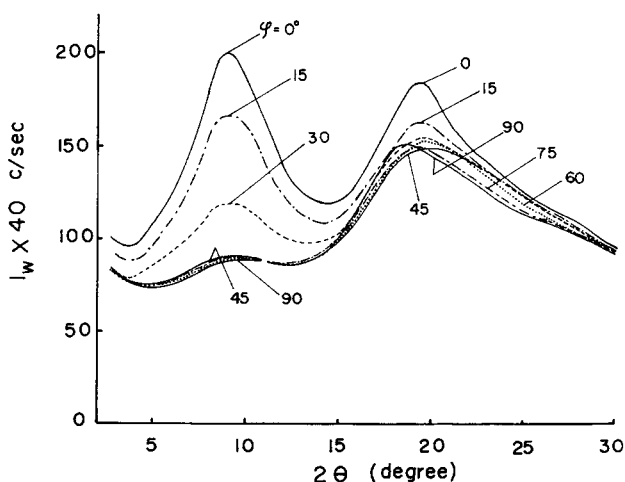


Fig. 6. Variation in scattering intensity I_w at various azimuthal angles for native fibers at 50% extension.

Similar appearances of the background intensity were observed for the grafted fibers over the whole range of reflection, except the reflection at the vicinity of $2\theta = 14^\circ$ (12° – 16°), where the minimum intensity was $\varphi = 90^\circ$.

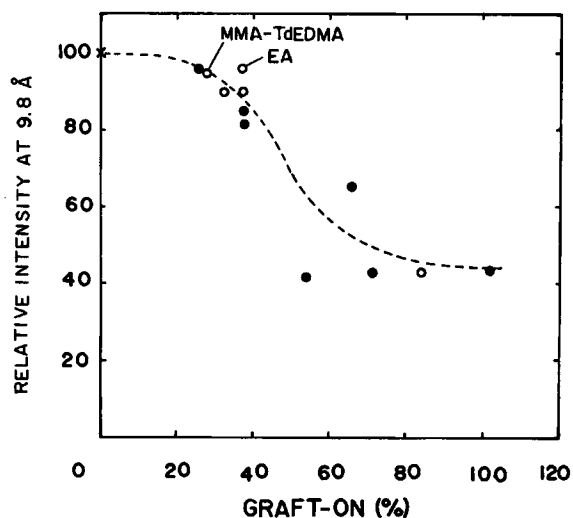
From the idea of the degree of crystallinity, the variation of the peak intensity at the 9.8 \AA and 5.1 \AA reflections, between the native and the grafted fibers at zero extension, was calculated from the intensities of the x-rays diffracted by the crystalline and amorphous phases. Estimation of the intensity arising from the latter was based on the line of the azimuthal intensity at $\varphi = 45^\circ$. The relative intensity from the α -crystalline phase of the grafted fiber is given by eqs. (11) and (12).

$$I_{\text{rel}} = \frac{i_{\varphi=0^\circ} - i_{\varphi=45^\circ}}{T \cdot f} \quad (\text{at the } 9.8 \text{ \AA} \text{ reflection}) \quad (11)$$

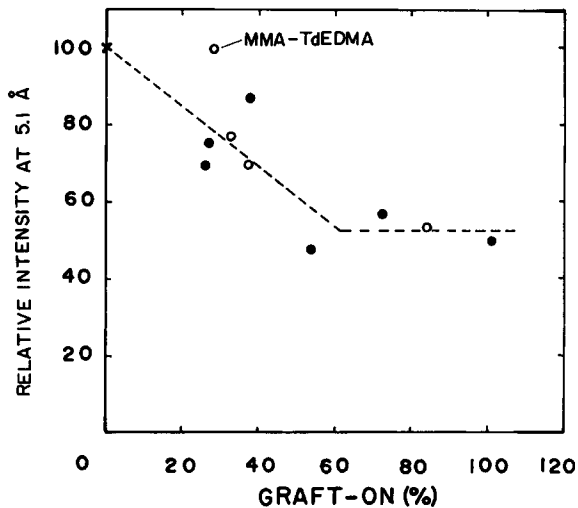
and

$$I_{\text{rel}} = \frac{i_{\varphi=90^\circ} - i_{\varphi=45^\circ}}{T \cdot f} \quad (\text{at the } 5.1 \text{ \AA} \text{ reflection}) \quad (12)$$

where i is the observed x-ray diffracted intensity from the specimen and f is the weight fraction of wool in the grafted fibers. The results are shown in Figure 7. Approximately similar behavior can be observed at 9.8 and 5.1 \AA reflections such as a sharply decreased relative intensity by about 50% of polymer introduced and a leveling off thereafter. It is apparent, therefore, that a considerable disruption of the components of the α -helix appears, although there is the complexity that the equatorial α -reflections resulted from the changes in lateral dimension of the microfibril by the deposition of polymer.



(a)



(b)

Fig. 7. Relative intensities at 9.8 Å equatorial reflection (a) and at 5.1 Å meridional reflection (b) vs. graft-on: (●) LiBr system; (○) NH₄Br system.

The Alpha-Beta Transformation in Keratin

There are two possible explanations for the mechanism of the disruption of α -material which should be considered: (1) The fact that the grafted polymer chains are linked to the low-sulfur proteins with average molecular weight²⁸ of 30,800 suggests that the growing polymer ends might be diffused in part into the α -phase. (2) When the polymer is deposited in the region which is chemically or physically laterally crosslinked, stress might be produced locally by the lateral swelling resulting from the deposition of

polymer. This stress might transfer to the fibril through the crosslinks and open up the α -helix. In either situation, the rigid polymer phase would prevent or restrict rotation of the polypeptide segments which had been deformed by the grafted copolymer or even act as an impurity in the polypeptide chain. As a result, it is most likely that these chains would be prevented from forming the β -chain crystal during stretching. The results obtained from the x-ray diffraction behavior of the grafted fiber support Bendit's hypothesis that β -material is produced in the noncrystalline phase of keratin. The proposed mechanism of the so-called α - β transformation is

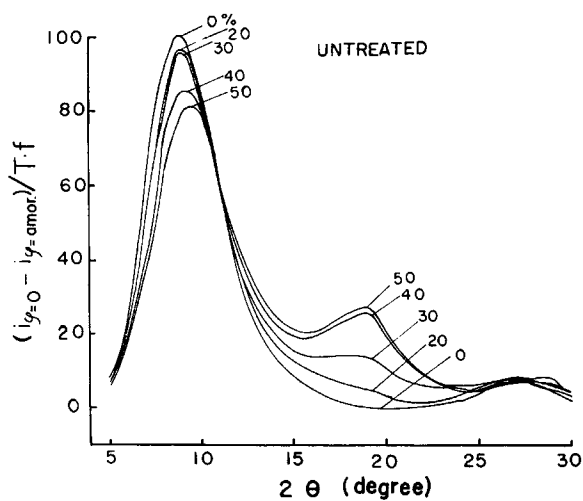


During the stretching process of the MMA-grafted wool fibers, prepared by the NH_4Br system in which over 30% polymer is present, no perceptible intensity change in the α -pattern and increase in intensity of the β -pattern were observed. Similar results were also obtained for the Lincoln wool fibers grafted by using the LiBr initiator system. Compared with the x-ray diffraction pattern for Merino wool, the structure of the β -reflections could be more clearly distinguished for the grafted Lincoln wool fibers with the initial intensity of the α -reflection remaining. These facts strongly suggested that the location of polymer differs greatly in each of the grafting systems and between the two types of cortex of the fibers.

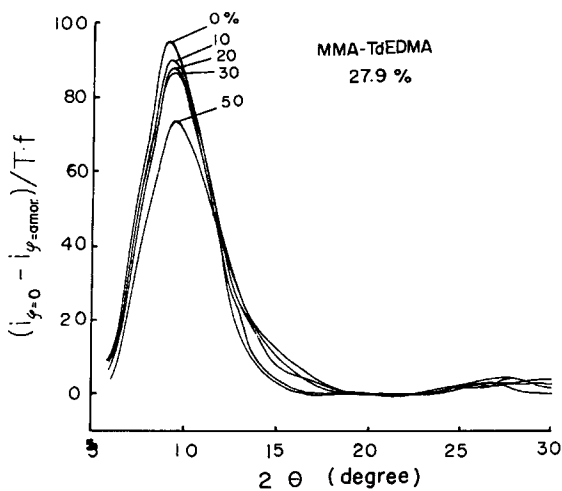
On the basis of electron microscopy, it has been postulated that the polymer is grafted predominantly in the orthocortex segments, especially in the aggregated material within the sheet structure and in the interstitial region of the microfibril. As a result of the hydrogen-bond breaking capacity of the LiBr system, the growing polymer chain can diffuse more easily into the matrix and interstitial materials. Only for the specimens prepared by the LiBr system was the reflected intensity near $2\theta = 14^\circ$ progressively increased with increase in the extension. These phenomena were observed for the grafted fibers containing over 25% of polymer, suggesting a specific interaction between the finely dispersed polymer and the polypeptide chains. When a low-swelling system such as aqueous NH_4Br solution is used for the grafting, polymer is likely to be located mainly around the protofibrils, but not in the aggregated materials. In such circumstances, interaction may arise to a great extent between the α -helix and the grafted polymer chains. This may bring about an increase in stability of α -helices against their unfolding. Even when the LiBr system was used for grafting, the stability of the α -pattern was increased by increasing the extent of grafting.²⁷

On the other hand, the grafted fibers having relatively low degrees of grafting with a polymer containing diethylene glycol groups gives no β -pattern during stretching processes. Figure 8b shows the characteristic changes of equatorial scattering intensities from the crystalline phase of α -keratin in the grafted fiber containing 27.9% of a copolymer of MMA with tetradecaethylene glycol dimethacrylate. The diffracted intensities at

the equatorial and meridional reflections from the α -crystalline phase of the grafted fibers were estimated to be, respectively, about 95% and 100% of those of the native fiber. This fact clearly suggests that little or no disruption of α -material occurs in the grafted fibers. At the 50% extended state of the grafted fiber, the intensity at 9.8 Å is reduced considerably, but is not correspondingly produced at 4.65 Å. This gives rise to an important suggestion for the origin of the β -structure, namely, that β -material might not be produced from the α -crystalline phase, but is predominantly produced from the noncrystalline phase, which is less stabilized by the chemical



(a)



(b)

Fig. 8 (continued)

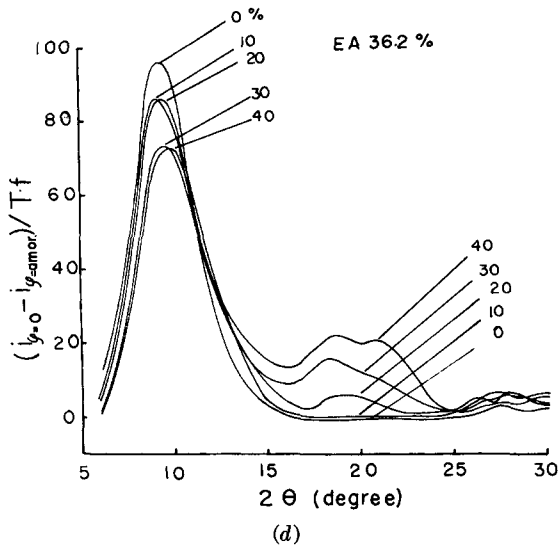
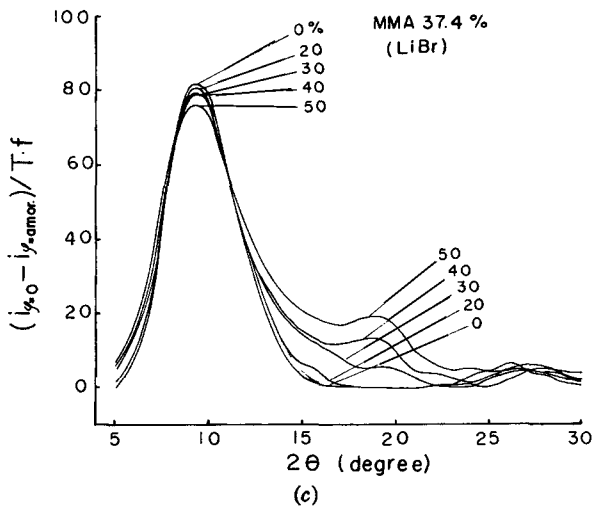


Fig. 8. Variation in equatorial intensity diffracted from crystalline phases at various extensions: (a) native wool fiber; (b) grafted fiber containing 27.9% copolymer of methyl methacrylate-tetradecaethylene glycol dimethacrylate (TdEDMA) prepared by NH_4Br system (1.0 g wool; 10 g NH_4Br ; 0.3 g $\text{K}_2\text{S}_2\text{O}_8$; 50 g BC; 34.7 g H_2O ; 5 g MMA; 10 mol-% to MMA, TdEDMA; 18°C; 20 hr); (c) 37.4% MMA graft (LiBr system; 18°C; 3 hr).

crosslinks and secondary bonds but which inhibits and retards the local deposition of polymer. The noncrystalline material must interact considerably with the hydrophilic groups of the copolymer. These experimental results also support the consideration that little possibility exists for the production of the β -chain crystal from the partly disrupted chains of α -crystallites resulting from the deposition of polymer.

Lipson and Speakman²⁹ reported previously that the β -pattern was not produced from the stretched fiber grafted with 33.5% methacrylic acid using the $\text{Fe}^{2+}-\text{H}_2\text{O}_2$ method. The location of the polymer may depend on the wool-monomer interaction since the acid monomer will reduce the interaction of the wool chains by causing salt-linkage breakdown. As a result, the grafted polymer chains may strongly interact with the molecules concerned with the production of β -material.

Figure 8c shows the reflection behavior in extension for the grafted fibers with 37.4% of MMA, initiated by the LiBr system at a low reaction temperature (18°C). The relative intensity at 9.8 Å is decreased by about 80%. A marked stabilization of the α -structure resulted from the applied polymer, but a considerable amount of the β -structure was generated by the extensions.

As a rule, little or no disruption of α -crystallites is produced by the deposition of a soft polymer such as poly(ethyl acrylate). Figure 8d illustrates that the variations in the reflected intensities from the crystalline phases of the grafted fibers with 36.2% ethyl acrylate are very similar to those of the native fibers during whole extension process. This, in turn, indicates that the polymer does not interfere with the α - β transformation. At 40% extension, a new diffraction peak appears at $2\theta = 21^\circ$, which is a characteristic reflection of the oriented polymer along the axis direction of the fibers.

Needless to say, M^* corresponds to the material present in the region which is not affected by the hydrophobic grafted copolymer. From the electron microscopy studies, it has been suggested that the grafted polymer is not located within the matrix between sheets for the sample grafted with 101.6% MMA prepared using the LiBr initiation system.¹⁸ Generally, it seems unlikely that β -material is produced from the matrix molecules be-

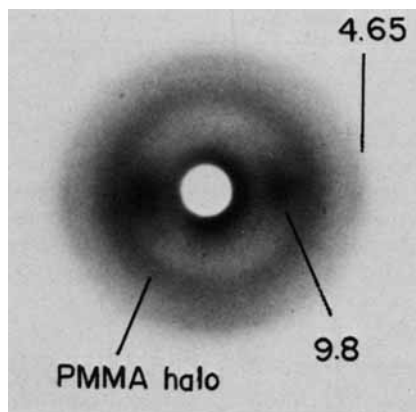


Fig. 9. X-Ray photograph at 50% extension for grafted Lincoln wool fiber containing 299% MMA prepared by two-step method. Grafting conditions: NH_4Br system; 30°C; 3 hr; 1st step—graft-on 46.2%; 2nd step—additional graft-on 252.8% after reduction treatment (0.1*N* thioglycollate) of the 1st grafts.

tween sheets in which disulfide crosslinks³⁰ and proline residues³¹ occur to any appreciable extent.

This postulation could be supported by the x-ray diffraction evidence of the 299% MMA-grafted sample prepared by successive grafting after a reduction treatment of the Lincoln wool fibers containing 46.2% of the polymer. As shown in Figure 9, a discrete β -pattern appears at 4.65 Å reflection on the diffraction photograph of the 50% stretched sample. This result suggests that the excessive polymer occurring in the high-sulfur regions of the matrix is not much concerned with the appearance of the β -pattern. This may also imply that β -material is present within a region where it has been surrounded and stabilized by the polymers during the first grafting process for unreduced condition of the fiber.

Recently, it has been found that the grafted polymer in Merino wool is linked to the protein chains with half-cystine contents of 576 μ mole/g protein, a value which is very similar to that of the low-sulfur group protein termed S-carboxymethyl keratine A. This suggests an identity of the materials occurring in the interstitial region of the microfibril and in the matrix within the sheets.²⁸ Deposition of polymer in the fiber, especially within these aggregates, must be greatly influenced by the reaction conditions used for the graft copolymerization, as observed with respect to the difference in the rate of grafting and the maximum level of grafting. When a system which swells less was used for the grafting at low reaction temperatures, the initial molecular conformation of the protein chain in some of those aggregates might well have been retained without being affected by the polymer deposition. However, further investigation is clearly needed for the determination of the origin of the β -structure.

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