

## Cooperative Unfolding of $\alpha$ -Keratin

M. FEUGHELMAN, *Division of Textile Physics,  
C.S.I.R.O. Wool Research Laboratories,  
Ryde, Sydney, Australia*

### Synopsis

The unfolding of the  $\alpha$ -helices of the microfibrils in  $\alpha$ -keratin fibers such as wool proceeds in a cooperative manner. When a wool fiber is extended in water, the load-extension curve obtained indicates on the basis of relationships derived from rate process theory, that the number of  $\alpha$ -helical residues  $n$  that unfold simultaneously is at least 50. This is probably an underestimation, as fiber diameter variability severely reduced any estimate of the value of  $n$ . Other experimental evidence is also indicated that support the existence of considerable mechanical cooperation between  $\alpha$ -helical components.

### Introduction

Considerable interest exists about the stability of the  $\alpha$ -helical state and the free energy difference between  $\alpha$  and  $\beta$  states of keratin fibers.<sup>1-9</sup> Certain of these estimates<sup>3,5,8</sup> for the difference of free energy between the folded and unfolded states of the polypeptide chains in keratin fibers range from 4.5 to 10.5 kcal./mole. These high values<sup>3,4,8</sup> were obtained because the measurements of stress on which the calculations were based were not equilibrium values. More recent estimates<sup>4,7</sup> place the value of the free energy difference at between 1 and 3 kcal./mole and possibly lower.<sup>6,7</sup> Diorio and others,<sup>6</sup> for example, claim that there is little or no free energy difference between the  $\alpha$  and  $\beta$  states in concentrated LiBr solution. Tompa<sup>10</sup> showed that heat evolved during  $\beta \rightarrow \alpha$  change in a 1:1 copolymer of DL-phenylalanine and DL-leucine was 1 kcal./residue. This means that, provided little entropy difference exists between the  $\alpha$  and  $\beta$  crystalline phases, the value of the free energy difference is also about 1 kcal./residue.

The side chains in  $\alpha$ -keratin, which represent about 50% of the structure, must play an important role in the stability of the  $\alpha$  and  $\beta$  configurations. Gratzer and Doty<sup>9</sup> have pointed out that the  $\alpha$ -helix in synthetic polypeptides is probably very stable *per se* but that the presence of side chains may reduce this stability. Hermans,<sup>12</sup> however, has recently shown that neither side-chain hydrogen bonding nor hydrophobic bonding plays an important role in stabilizing the helices of polylysine and poly(glutamic acid). In the case of  $\alpha$ -keratin it is the presence of bulky side chains which, according to Crick,<sup>11</sup> results in a coiled-coiled arrangement of the  $\alpha$ -helices rather than the side-by-side relationship which exists for such artificial polypeptides as

poly- $\gamma$ -methyl-L-gutamate. The presence of disulfide bonds in  $\alpha$ -keratin also assists in stabilizing the structure. Their presence, as has been suggested by Haly,<sup>13</sup> maintains the cooperative extension of the folded  $\alpha$ -keratin structure by maintaining the phase relationship between the  $\alpha$ -helices forming the microfibrils.

The unfolding of the  $\alpha$ -helical components during extension of keratin fibers such as wool and hair occurs, due to the presence of the aforementioned sidewise stabilization, as a cooperative event. That is, complete groups of  $\alpha$ -helices unfold simultaneously. Ciferri<sup>4</sup> in examining the load-extension curves of single wool fibers in water at various temperatures regarded the extension of a fiber into the yield region ( $>2\%$  strain) as unfolding of  $\alpha$ -crystallites into extended  $\beta$ -crystallites. He applied thermodynamic theory in his analysis which regards the phase change from  $\alpha$ - to  $\beta$ -keratin as involving a very large number of helices in each crystallite, all unfolding concurrently. Others,<sup>3</sup> using rate process theory, have considered the unfolding of  $\alpha$ -helices as individual events not necessarily acting in a cooperative manner. In this paper, rate process theory has been applied to events occurring cooperatively with the number of helices cooperating having a definite value in between the limits already discussed, namely  $n = \infty$  in the case of thermodynamic approach, and  $n = 1$  for the case of rate process theory applied to unfolding helices considered as individual events.

### "Cooperative" Two-State Model

Let us consider a fiber consisting of a continuum of two-energy-state units of the type described by Burte and Halsey.<sup>14</sup> Let the states A and B of these units have a free energy difference  $\Delta F^*$  per gram molecule of units and let the free energy barrier between the two states be  $\Delta F^* + \Delta F$  (see Fig. 1). The state A is the folded form and state B the extended form of the units.

We define

$$K = (kT/h) \exp \{ -\Delta F/RT \}$$

and

$$D = \exp \{ -\Delta F^*/RT \}$$

where  $R$  is the universal gas constant,  $T$  is absolute temperature,  $k$  is Boltzmann's constant and  $h$  is Planck's constant.

If  $n$  is the fraction of units in the unfolded form, and a stress  $f$  is acting on the fiber, then the rate of unfolding of the units is given from the work of Burte and Halsey<sup>14</sup> by

$$dr/dt = (1 - r)KD \exp \{ 2\mu\alpha f \} - rK \exp \{ -2(1 - \mu)\alpha f \} \quad (1)$$

where  $\alpha = V/2kT$  for an asymmetrical energy barrier between the A and B states,  $V$  is the volume of the flow hole per two-state unit in the change from

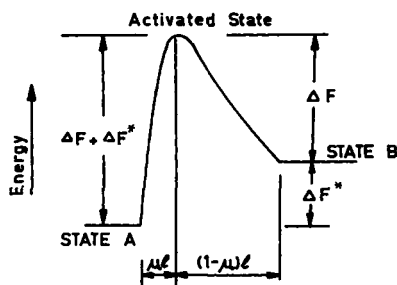


Fig. 1. Diagrammatic representation of the relationship between the free energies of a Burte-Halsey unit in the folded state A, extended state B, and the activated state. The distance swept out in going from state A to B is  $l$ ;  $\mu l$  is the distance from A to the activated state, and  $(1 - \mu)l$  from the activated state to state B.

state A to state B, and  $\mu V$  is the flow hole volume swept out by a unit in going from state A to the activated state. For a symmetrical energy barrier,  $\mu = 1/2$ .

In eq. (1) it is assumed that each two-state unit can unfold without the cooperation of its neighbors. If, however,  $n$  units in parallel are bound together in some way so that they must unfold and fold simultaneously, then eq. (1) must be modified to

$$dr/dt = (1 - r)[KD \exp \{2\mu\alpha f\}]^n - r[K \exp \{-2(1 - \mu)\alpha f\}]^n \quad (2)$$

since the probability of a group  $n$  unfolding or folding would be changed by the power  $n$  relative to the probability of a single unit unfolding or folding. At equilibrium, when  $dr/dt = 0$ .

$$r = \frac{1}{1 + (D^{-1} \exp \{-2\alpha f\})^n}$$

i.e.,

$$r = \frac{1}{\exp \{n[(\Delta F^*/RT) - 2\alpha f]\} + 1}$$

from which

$$f = \frac{\Delta F^*}{2\alpha RT} - \frac{1}{2n\alpha} \ln \left( \frac{1 - r}{r} \right) \quad (3)$$

It should be noted that  $f$  in eq. (3) is independent of the asymmetry of the energy barrier. For  $r = 0.5$ ,  $f$  is independent of  $n$  and equal to  $\Delta F^*/2\alpha RT$ . Substituting  $\alpha = V/2kT$ , and  $r = 0.5$ ,  $f = \Delta F^*/NV$ , where  $N$  is Avogadro's number.

If a fiber consists of a continuum of two-state units, then the value of  $r$  is proportional to the strain for a given stress  $f$  and may be equated to the strain. In Figure 2 the values of  $r$  for  $n = 1, 3, 10, 30$ , and  $\infty$  are shown plotted against values of  $f/f_0$ , where  $f_0$  is the stress on the fiber at  $r = 0.5$ .

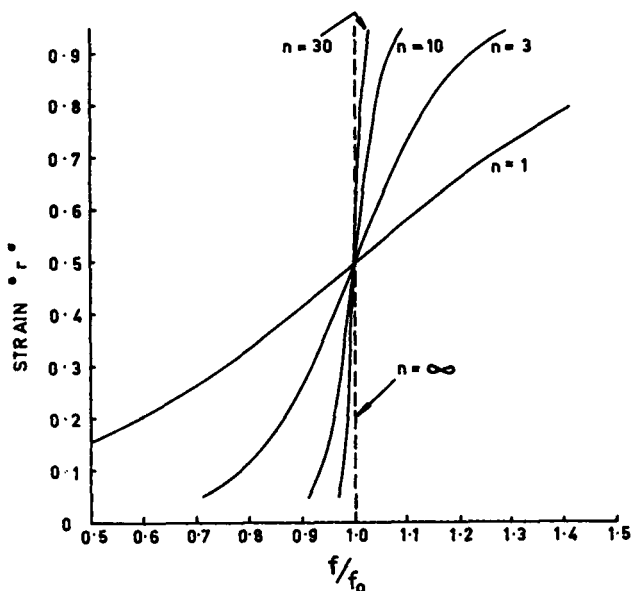


Fig. 2. Relationship between the strain factor  $r$  and the force  $f/f_0$  acting on a continuum of two-state units which unfold and refold cooperatively in groups of  $n$  for values of  $n = 1, 3, 10, 30$ , and  $\infty$ .

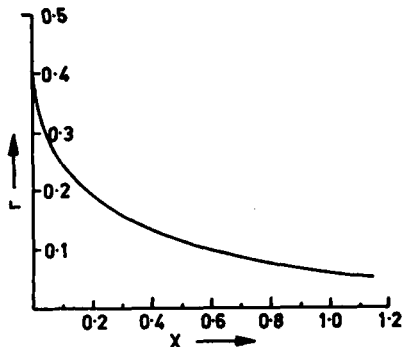


Fig. 3. Plot of  $X$  against  $r$ , where  $X = 4r - 2 \ln [(1 - r)/r]$ .

The value of  $\Delta F^*$  is taken as 1.8 kcal./g.-mole. For large values of  $n$ , for all values of strain  $r$  between 0 and 1

$$f = f_0 = \Delta F^*/NV$$

From eq. (3)

$$f/f_0 = 1 - (1/2n\alpha f_0) \ln [(1 - r)/r] \quad (4)$$

From eq. (4) the slope of the tangent to the curve at  $r = 0.5$  is given by

$$\frac{1}{f_0} \left. \frac{df}{dr} \right|_{r=0.5} = \frac{2}{n\alpha f_0} \quad (5)$$

Equation (5) leads to one estimate for the value of  $n$ , the number of units unfolding concurrently, when an  $\alpha$ -keratin fiber such as wool or hair is extended. This value however, as will be shown, can, due to the effects of the cross-sectional area variability of the fiber, result in an underestimation. A better estimate can be made by measurement of the deviation from linearity of the curves in Figure 2 as the value of  $r$  differs from  $r = 0.5$ .

The tangent at  $r = 0.5$  and  $f = f_0$  to the curve of eq. (4) is given by

$$f/f_0 = (2/n\alpha f_0)(r - 0.5) + 1 \quad (6)$$

Thus the difference between the line expressed in eq. (6) and the curve in eq. (4) for the value of  $f/f_0$  at any value of  $r$  is given by

$$\Delta \left( \frac{f}{f_0} \right) = \frac{1}{2n\alpha f_0} \left| 4r - 2 + \ln \left( \frac{1-r}{r} \right) \right| \quad (7)$$

We set  $X = 4r - 2 + \ln [(1-r)/r]$

A plot of  $X$  versus  $r$  is shown in Figure 3. The eq. (7) may be re-expressed as

$$\Delta(f/f_0) = (1/n)(X/2\alpha f_0) \quad (8)$$

Equation (8) gives another method of estimating the value of  $n$ .

### Application to the $\alpha$ -Keratin Fiber

The load-extension curves in water of  $\alpha$ -keratin fibers such as wool and hair have three distinct regions as shown in Figure 4. Once the fiber is decrimped there is the initial stiff region known as the Hookean region (AB), followed beyond about 2% strain by the yield region (BC) and the post-yield region (CD). For extensions beyond the end of the Hookean region (B), the  $\alpha$ -keratin phase is continuously reduced by unfolding and  $\beta$ -keratin is formed. The transition from the Hookean to the yield regions at  $\sim 2\%$  strain is quite sharp even for load-extension curves taken at nearly equilibrium conditions (see Fig. 4). The change in slope between the two regions is of the order of two decades. Considering the trend indicated in the load-extension curves in Figure 2, this sharpness of transition in the load-extension curve occurring at around 2% extension indicates a high value of  $n$ , that is, cooperation of folding and unfolding of the  $\alpha$ -helices.

In estimating the value of  $n$ , the number of residue units of the  $\alpha$ -helices acting cooperatively during the extension of an  $\alpha$ -keratin fiber such as wool or horse hair in water, it is necessary both in using eqs. (5) or (8) to have an estimate of the value of  $\alpha f_0$ , where  $\alpha = V/2kT$ .  $V$ , the volume swept out by one unit, in this case for an  $\alpha$ -helix residue going from an  $\alpha$  to a  $\beta$  phase, has been shown to be<sup>7</sup>  $200 \times 10^{-24}$  cc.,  $k = 1.38 \times 10^{-16}$  ergs/degree, and  $T = 293^\circ\text{K}$ .; thus  $\alpha = 1.84 \times 10^{-9}$  cc./erg. Various estimates<sup>7,15</sup> may be made of the value of the equilibrium force  $f_0$  between  $\alpha$  and  $\beta$  phases at  $r = 0.5$  for fibers in water. These values are in the range  $1.5 \times 10^8$ – $2.0 \times 10^8$  dynes/cm.<sup>2</sup>. Taking  $f_0 = 1.8 \times 10^8$  dynes/cm.<sup>2</sup>, we have

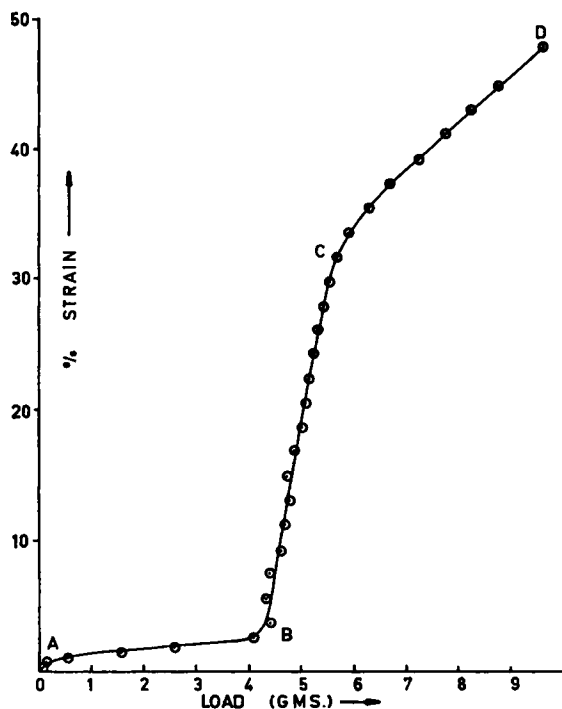


Fig. 4. Typical load-extension curve in water at 20°C. for a Corriedale wool fiber taken over a period of 25 days. From the origin to the end of the Hookean region (*B*) the rate of extension was  $\frac{3}{8}\%$ /hr. and beyond the Hookean region (*B*, *C*, *D*) the rate of extension was  $1\frac{7}{8}\%$ /day.

$\alpha f_0 = 0.33$ . This value of  $\alpha f_0$  is based on the assumption that all the fiber unextended consists of  $\alpha$ -helices. Actual estimates of helix content of  $\alpha$ -keratin fibers range from 0.2 to 0.5. This means that a corrected value of  $\alpha f_0$  is approximately 1.

Let us consider the  $\alpha$ -helices present in a wool fiber responsible for the extension of the yield region only, that is for extensions up to about 30%. These helices, although they form only a fraction of the total of  $\alpha$ -helical content of a wool fiber, form the group responsible for the slope of the yield region and from the point of view of analysis for those units,  $r = 1.0$  for the extension of about 30%. All the results of the eqs. (1) to (8) may be considered to apply to this group of units. For normal wool fibers the value of yield region slope/ $F_{15}$  is approximately unity, where  $F_{15}$  is the stress at 15% extension; that is,

$$\frac{1}{f_0} \left. \frac{df}{dr} \right|_{r=0.5} = 0.3$$

Substituting in eq. (5) yields  $n = 1/0.15 \approx 6$ .

This, however, is an underestimation caused in part by the nonuniformity of normal wool fibers. Collins and Chaikin<sup>16</sup> have shown the ef-

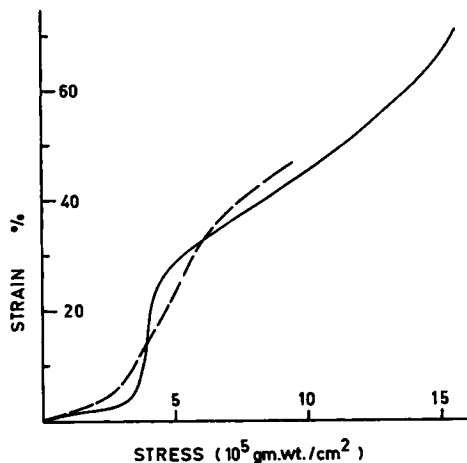


Fig. 5. Comparison of the nonuniform (coefficient variation 20%) and uniform wool fiber stress-strains in water: (1) uniform fiber, (2) nonuniform fiber. Data of Collins and Chaikin.<sup>16</sup>

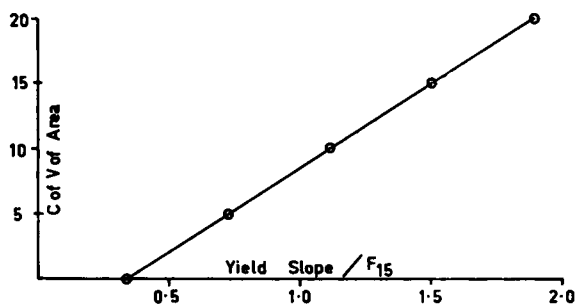


Fig. 6. Relationship between the coefficient of variation of the cross-sectional area of a wool fiber and measured values of the ratio of yield region slope to force at 15% extension ( $F_{15}$ ). The relationship was plotted from the data of Collins and Chaikin.<sup>16</sup>

fect of this nonuniformity on the load-extension curve of single wool fibers in water (see Fig. 5) and give a relationship between the values of yield region slope/ $F_{15}$  for varying values of the coefficient of variation of the cross-sectional area of their fibers (see Fig. 6). From their results a better estimate of yield region slope/ $F_{15}$  is 0.33, which means that  $n = 1/0.05 = 20$ .

The criterion of deviation from linearity of the equilibrium load-extension curve expressed in eq. (8) appears less sensitive to nonuniformity of the fiber than eq. (5).

Examination of curves such as in Figure 4 suggests that the deviation  $\Delta(f/f_0) \approx 0.01$  for  $r$  between 0.05 and 0.07. This means that from the curve in Figure 3 that  $X \approx 1$ . Equation (8) then reduces to  $0.01 = \frac{1}{2}(1/n)$ , that is,  $n = 50$ .

Ruoff and Eyring,<sup>3</sup> in considering the unfolding of the  $\alpha$ -helices in human hair obtained a value for the volume of the flow hole of  $8150 \times 10^{-24}$  cc.

In their analysis they considered one unit unfolding at one time. If we compare this value with that of  $200 \times 10^{-24}$  cc. for the flow-hole volume swept out by an  $\alpha$ -helix residue, their value of  $8150 \times 10^{-24}$  cc. may be interpreted to mean that the number of units unfolding cooperatively is about 40, a value for  $n$  within the range indicated above.

However it must be recognized that the estimates being carried out are for  $1/n$ , which becomes progressively more difficult for large values of  $n$ . The correct value of  $n$  may be many orders greater than 50, but the methods developed here because of effects of fiber variability, would not be capable of resolving this point.

### Further Evidence on Cooperative Unfolding

Haly<sup>17</sup> and others have shown that the helices in keratin fibers immersed in lithium bromide solutions of varying molarity go through a helix-coil transition in the concentration range of 6.2–6.6*M*. This transition was observed by using x-ray diffraction, optical birefringence, and fiber length as indicators of the change. The transition is quite sharp, indicating again the cooperative nature of the unfolding of the  $\alpha$ -helices.

Another indication of the high degree of cooperation in the unfolding of the  $\alpha$ -keratin phase comes from the study of the fracture mechanism in wool fibers. Mason<sup>18</sup> has shown that, when a sharp notch is cut into a wool fiber (Fig. 7*a*) and the fiber is extended at 65% R.H. and room temperature, the crack propagation occurs parallel to the fiber direction for many millimeters (Fig. 7*b*) until a natural flaw is reached, which allows crack propagation across the fiber section. This behavior can be understood in terms of the two-phase model for wool fibers in which the fiber may be considered to consist of microfibrils embedded in a noncrystalline matrix (Fig. 7*c*). At 65% R.H. the matrix behaves as a glass. The microfibrils, consisting of  $\alpha$ -helices with side-to-side stabilization, require cooperative

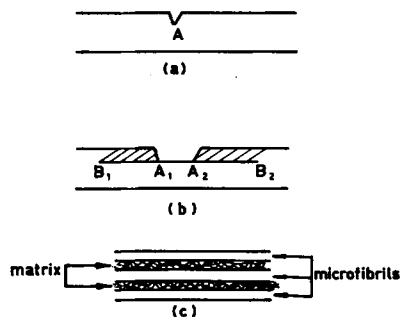


Fig. 7. Process of flaw propagation in a wool fiber at 65% R.H.: (a) fiber notched at point A; (b) fiber extended. (The flaws are propagated in the fiber direction, i.e.,  $A_1B_1$  and  $A_2B_2$ , the shaded portions of the fiber are unextended resulting in a widening of the formed notch at A to  $A_1A_2$ ); (c) representation of the relationship between the crystalline microfibrils and the glassy matrix inside the fiber, the flaw propagation being confined to the matrix.



unfolding for any extension. Under these conditions any fracture mechanism to cut across a microfibril would have to simultaneously break a large number of bonds. The mechanical cooperation between the  $\alpha$ -helices in the microfibrils forces crack propagation to be confined to the matrix and hence in the fiber direction unless a natural flaw exists in the structure.

### Comparison of Results for the Rate Process and Thermodynamic Theories

For large values of  $n$ , folded units in state A and unfolded units in state B can coexist in equilibrium only at a value of external stress given by  $f = f_0 = \Delta F^*/VN$ , irrespective of the fraction  $r$  of units in state B that are present. Ciferri,<sup>4</sup> using basic thermodynamic arguments in considering the transition at a constant temperature of a keratin fiber going from a folded crystalline phase  $\alpha$  to a crystalline phase  $\beta$ , showed that the two phases can coexist in equilibrium at a fixed force  $F$  on the fiber. Let  $\Delta L$  be the length change of the entire fiber when changed from being completely in the  $\alpha$  phase to  $\alpha$  phase  $\rightleftharpoons$   $\beta$  phase equilibrium at a constant temperature and pressure and a force  $F$  on the fiber. Then the difference of free energy  $\Delta G$  for the fiber between the states  $\alpha$  and  $\beta$  is given by work done on the system going from  $\alpha$  to  $\beta$ , i.e.,  $\Delta G = F\Delta L$ , and

$$F = \Delta G/\Delta L \quad (9)$$

Thus  $\Delta G = \Delta F^*$  when the experiments are carried out at atmospheric pressure, and  $F/A$  is the stress on the fiber.

If the cross-sectional initial area of the fiber is  $A$ , then the number of units which go from  $\alpha$  to  $\beta$  as the fiber is extended  $\Delta L$  is  $A\Delta L/V$ . (This assumes the whole fiber is  $\alpha$  or  $\beta$ .)

If the number of units which go from  $\alpha$  to  $\beta$  corresponds to 1 g.-molecule of material, then  $N = A\Delta L/V$ ; that is, from equation (9)

$$F/A = \Delta G/VN \quad (10)$$

It follows that eq. (10) is identical with the equation for stress  $f = \Delta F^*/VN$  obtained from rate process theory for  $n$  large. The advantage of applying rate process theory lies mainly in that estimates may be made of the number of units  $n$  involved simultaneously in the unfolding or folding process. Further, if necessary, eq. (2) for  $dr/dt \neq 0$  enables nonequilibrium situations to be considered.

### Concluding Remarks

Quite a number of experiments point to the necessity of cooperative unfolding of the  $\alpha$  phase in the intact  $\alpha$ -keratin fiber. This necessity may arise from the environment in the fiber external to the  $\alpha$ -helices. Haly's results<sup>13</sup> on reduced disulfide fibers, for example, suggest that the cystine link is involved in maintaining lateral mechanical cooperation between the helices. In solution,<sup>19</sup> the unfolding of  $\alpha$ -helices of helical extracts of keratin has shown to occur over a widespread temperature range (35–80°C.).

This contrasts with the sharp approach to melting of the  $\alpha$ -helices for fibers in water.<sup>20</sup> Again, this latter suggests a high degree of cooperation in the unfolding of the  $\alpha$  phase in the intact fiber. In solution, however, in the absence of environmental restrictions which exist inside the fiber, the co-operation between the unfolding of  $\alpha$ -helices may be considerably reduced, resulting in a very wide temperature range over which helix unfolding occurs.

The calculations based on rate process theory suggest that at least 50  $\alpha$ -helix residues are involved cooperatively in the unfolding process. This is likely to be an underestimation due to the intrinsic experimental errors involved. It does, however, suggest that units of the  $\alpha$  phase, that is portions of the microfibrils, unfold simultaneously. This means that models<sup>21</sup> suggesting the formation of mixed phases of  $\alpha$  and  $\beta$  existing side by side when the fibers are extended appear improbable. It also indicates that, in the consideration of the transformation in  $\alpha$ -keratin in which unfolding of the  $\alpha$  phase is involved, we should consider the fiber as consisting of units of  $\alpha$ -helices together with their environment, which plays a vital stabilizing role. These units would involve portions of the microfibrils and some of the neighboring matrix, and in any unfolding process it is the stability of these units that should be considered rather than that of the individual  $\alpha$ -helix.

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### Résumé

Le déplissement d'hélices  $\alpha$  de microfibrilles au sein de fibres d' $\alpha$ -kératine telle que la laine résulte d'un processus coopératif. Lorsque la fibre de laine est étirée dans l'eau, la courbe poids-extension obtenue indique sur la base d'un rapport dérivé de la théorie du processus de vitesse que le nombre de résidus  $\alpha$ -hélicoïdaux  $n$  qui se déplissent simultanément est au moins égal à 50. Ceci est probablement une sous-estimation vu que la variabilité de la fibre réduit très fortement toute estimation de la valeur de ce même nombre  $n$  autres évidences expérimentales sont également indiquées qui montrent l'existence d'une coopération mécanique considérable entre les composants de l'hélice- $\alpha$ .

### Zusammenfassung

Die Auffaltung der  $\alpha$ -Helix der Mikrofibrillen in  $\alpha$ -Keratinfasern wie Wolle verläuft als kooperativer Prozess. Bei Dehnung einer Wollfaser in Wasser zeigt die erhaltenen Belastungs-Dehnungskurve mit den aus der Theorie der Geschwindigkeitsprozesse abgeleiteten Beziehungen, dass die Anzahl der  $\alpha$ -Helixeinheiten  $n$ , die sich gleichzeitig auffalten zumindest 50 beträgt. Dieser Wert ist wahrscheinlich zu niedrig, da die Streuung des Faserdurchmessers den abgeleiteten Wert von  $n$  stark herabsetzt. Es werden weitere experimentelle Befunde mitgeteilt, die das Bestehen eines beträchtlichen mechanischen Zusammenwirkens der  $\alpha$ -Helixkomponenten zeigen.

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