STATISTICAL-MECHANICAL STUDIES OF
THE $\alpha \rightleftarrows \beta$ TRANSFORMATION IN KERATINS

II. The Tension-Length Isotherms

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ABSTRACT   In attempting to understand the yield region of the $\alpha \rightleftarrows \beta$ transformation in keratins (Astbury and Woods, 1933), we recently proposed a statistical-mechanical model (David and Schor, 1965) which generalized the work done by others on the helix $\rightleftarrows$ random coil transformation (Zimm and Bragg, 1959; Gibbs and DiMarzio, 1959) (thermal denaturation) to the case of a polypeptide under external tension (Birstein, 1962). We wish now to report the comparison of the quantitative aspects of this model to the observed tension-length isotherms (in the yield region) of Cotswold wool.

As in our previous work (David and Schor, 1965) let us consider an isolated α helix in which there are n_α short or (α) residues of length ℓ_α and n_β long or (β) residues of length ℓ_β, $(\ell_\beta \simeq 2\ell_\alpha)$ under an external tension τ in the helical axis direction. Let L represent the length of the chain and N, the total number of residues:

$$L(N, T, \tau) = n_\alpha(N, T, \tau)\ell_\alpha + n_\beta(N, T, \tau)\ell_\beta , \tag{1}$$

$$N = n_\alpha + n_\beta . \tag{2}$$

Equations 1 and 2 give the tension-length isotherms in terms of the equilibrium values of n_α and n_β.

In order to determine the equilibrium values of n_α and n_β, it is necessary to introduce a model for the polypeptide chain. Following Zimm and Bragg we assume that a given state of the polypeptide chain can be completely determined by the state of the oxygen atoms alone and that in the α helix phase the oxygen atom is bonded to the hydrogen atom of the third preceding segment. The state of the chain is then completely determined by whether or not each segment is bonded (denoted by α). Unbonded oxygens are denoted β. In the present context, the bonded or α state corresponds to a short residue, and the unbonded or β state corresponds to a long residue.

The statistical weight of a given state of the chain is taken by Zimm and Bragg to be the product of the following factors: (a) the quantity 1 for every β residue in the chain, (b) the quantity s for every α residue in the chain, (c) the quantity σ for every α residue that follows a β residue in the chain, (d) the quantity 0 for every α residue that follows a number of β residues less than μ. In order to generalize the treatment of Zimm and Bragg to include the effect of the external tension we have introduced the Boltzmann factor:

$$z = \exp\left(-\tau(\ell_\beta - \ell_\alpha)/kT\right), \tag{3}$$

where $\tau(\ell_\beta - \ell_\alpha)$ is the work done to extend a residue from length ℓ_α to ℓ_β, and kT is the thermal energy. For a helix under an external tension τ in the direction of the helix axis we should replace s by sz in the above assignments of the statistical weights. The factor $s(T)$ measures the contribution to the partition function of a bonded segment relative to that of an unbonded segment for the case of 0 tension. The parameter σ represents a nucleation parameter which is a measure of the difficulty of initiating a new helical (bonded) section of the chain. This parameter allows us to treat the three-dimensional problem in a one-dimensional manner. The parameter μ also represents an interaction effect between adjoining segments of the chain; the larger the value of μ the sharper the transition.

For the case $\mu = 3$ we have verified the results of Applequist (1963) and Zimm and Bragg, that the fractional number of hydrogen bonds in the polypeptide at equilibrium is given by:

$$\frac{\langle n_\alpha \rangle}{N} = \frac{1}{N-3}\left[\frac{\partial \ln Q}{\partial \ln sz}\right]_\sigma = \frac{1}{N-3}\frac{sz}{Q}\sum_1^4\left\{\frac{\partial Q}{\partial \lambda_i}\frac{\partial \lambda_i}{\partial sz}\right\}_\sigma + \left\{\frac{\partial Q}{\partial sz}\right\}_\sigma, \tag{4}$$

where the partition function Q is given by:

$$Q = \sum_{i=1}^4 \frac{\lambda_i^{(N+2)}(\lambda_i - sz)}{4(\lambda_i^3 - 3(1+sz)\lambda_i + 2sz)}, \tag{5}$$

and the $\{\lambda_i\}$ are the distinct eigenvalues of the statistical weight matrix M:

$$M = \begin{matrix}
1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
sz\sigma & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & sz & 0 & 0 & 0 & sz & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & sz & 0 & 0 & 0 & sz
\end{matrix} \tag{6}$$

In order to fit the experimental tension-length isotherms to the theoretical isotherms in the yield region, it is necessary to choose s and σ values for each isotherm. The relevant parameters for these isotherms are given in Table I. The entries in the first and second columns of Table I were determined by estimating the midpoint of the yield region. In computing z values at the midpoint of the yield region we have assumed that the tension is uniformly distributed across the cross-section of the fiber. The z values were then calculated using the value of 10 A for the diameter of the α helix and a value of 1.5 A for $\ell_\beta - \ell_\alpha$. The s values for the isotherms were obtained from the condition that the product of s and z at the midpoint of the transition region equals 1, i.e., the experimental and calculated values of the percentage extension were fitted to agree at the midpoints of the transition. The yield region was then

TABLE I

PARAMETERS FOR TENSION-LENGTH ISOTHERMS FOR COTSWOLD WOOL
IN WATER*

T	f at midpoint of yield region, initial area	Elongation at midpoint of yield region	z at midpoint of yield region‡	s	σ	Average % deviation
°C	g/cm^2	%				
0	6.3×10^5	16.0	0.145	6.90	0.015	6.3
24.4	5.5	16.3	0.212	4.70	0.013	8.0
32.9	4.9	16.5	0.262	3.81	0.010	7.6
53.8	4.6	16.8	0.308	3.24	0.009	2.7
64.0	4.3	17.0	0.344	2.90	0.005	9.5
75.2	4.2	23.0	0.369	2.71	0.004	9.0
84.7	3.8	26.5	0.413	2.42	0.002	12.7
92.0	3.2	29.5	0.481	2.08	0.002	17.5

* Speakman, 1929.
‡ The notation is from David and Schor, 1965.

expanded to 100% extension by assuming that the experimentally observed percentage elongation at the midpoint of an isotherm corresponds to an extension of 50% for the *extensible helical regions* at these comparatively low tensions. This scaling of the tension-length isotherms is thus equivalent to the physical assumption that only a fraction ($\simeq 30\%$) of the residues in the individual α helices are extensible in the yield region, but that these are able to undergo an approximately twofold reversible extension. For example, for the 0°C isotherms we have multiplied the percentage elongation by the scale factor $50/16.25 = 3.08$, and similarly for other isotherms. Then, using Equation 4, we have fit the theory to the data.

The values of σ which give good fits to the experimental data are listed in the sixth column of Table I. These values were obtained by trial and error. Having produced "theoretical" isotherms, we then divided these isotherms by their appropriate scale factors. The results of these computations for selected isotherms are shown in

Fig. 1. Five or six computed points were used on each isotherm. The average absolute percentage difference between the calculated and experimental points is shown in Table I. The average absolute percentage difference between the calculated and the observed elongations for the eight isotherms is 9.2%.

The parameter s was assumed to vary according to the equation:

$$s = s_0 \exp\left(-\epsilon/kT\right), \tag{7}$$

where ϵ is an effective hydrogen-bonding parameter, k is Boltzmann's constant, and T is the temperature in °K. Equation 8 gives the best parameters for s_0 and ϵ/k obtained by the method of least squares:
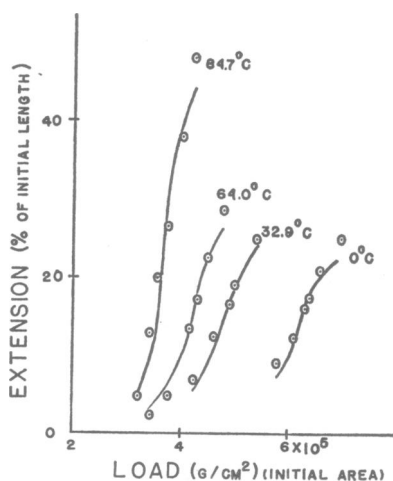
$$s = 0.081 \exp\left(1202/T\right). \tag{8}$$

FIGURE 1 A comparison of typical experimental tension-length isotherms for Cotswold wool in water with those predicted by the model. The parameters used were $N = 400$ and $\mu = 3$. The solid lines represent the predicted isotherms.

Using Equation 8 a value of approximately 2.4 kcal/mole has been obtained for ϵ. The temperature at which α helices should convert to the extended β form in the absence of tension, i.e. $s = 1$, is approximately 203°C.

The variation of σ with temperature is approximately linear. Equation 9 gives the results obtained by the method of least squares:

$$\sigma = 0.058 - 1.53 \times 10^{-4}T, \tag{9}$$

where T is in °K.

If it is assumed that all the tension is applied to the α helices only and none to the matrix which occupies approximately two-thirds of the cross-sectional area of the fiber, we obtain:

$$s = 2.5 \times 10^{-4} \exp\left(3961/T\right), \tag{10}$$

and $\epsilon = 7.9$ kcal/mole. Table II presents a summary of the results for $s(T)$, ϵ, and the temperature at which α helices should convert to the extended or "β" form in the absence of tension ($s = 1$) for various reasonable values of $l_\beta - l_\alpha$ and the effective diameter D of the α helices.

Having assumed that the elastic properties of Cotswold wool in water in the yield region are primarily due to the unfolding of individual α helices in the protofibrils, and using the statistical model of David and Schor (and Birstein), good quantitative agreement for the tension-length isotherms in this region have been obtained with reasonable assumed values for the chain parameters.

The "nucleation parameter" σ varies linearly with temperature decreasing at the higher temperature as should be expected. The magnitude of σ is roughly 100 times as great as has been obtained for α helices in solution (Rifkin and Applequist, 1964; Zimm, Doty, and Iso, 1959). These relatively high values for σ we believe to be a

TABLE II

EFFECTIVE HYDROGEN-BONDING ENERGY ϵ AS A FUNCTION OF
VARIOUS α HELIX PARAMETERS

$l_\beta - l_\alpha$	D	$s(T)$	ϵ	T for $s = 1$	
A	A		kcal/mole	°C	
1.5	10	$8.1 \times 10^{-2}e^{1202/T}$	2.4	206	Tension assumed to be
1.5	11	$4.8 \times 10^{-2}e^{1466/T}$	2.9	206	applied uniformly to the
1.8	10	$4.9 \times 10^{-2}e^{1444/T}$	2.9	206	cross-section of the fiber
1.8	11	$2.6 \times 10^{-2}e^{1743/T}$	3.5	206	
1.5	10	$2.5 \times 10^{-4}e^{3961/T}$	7.9	205	Tension assumed to be
1.5	11	$4.7 \times 10^{-5}e^{4795/T}$	9.5	205	concentrated at the pro-
1.8	10	$4.8 \times 10^{-5}e^{4783/T}$	9.5	205	tofibrils
1.8	11	$6.4 \times 10^{-6}e^{5741/T}$	11.4	205	

result of the fact that interactions with neighboring helices in the protofibrils and the matrix considerably broaden the transition in the fiber compared to the transition observed in dilute solutions. The fact that σ values are nevertheless much less than 1 lends support to the assumption of the cooperative nature of the phenomenon.

A reasonable exponential variation for $s(T)$ has been obtained. The functional dependence of $s(T)$ and $\sigma(T)$ are sensitive to the values chosen for the α helix parameters $l_\beta - l_\alpha$ and D, and to the assumed distribution of tension across the cross-section of the fiber. The order of magnitude obtained for the effective hydrogen-bonding parameter ϵ, however, is less sensitive to the detailed assumptions. An inspection of Table II shows that the range of values obtained for ϵ is 2.4–11.4 kcal/mole. This result compares favorably with the estimate of Pauling of approximately 8 kcal/mole (Pauling, Corey, and Branscomb, 1951). The fact that the hydrogen-bonding parameter ϵ as determined from an analysis of the tension-length isotherms agrees with independent theoretical estimates seems reasonable on the

basis of the postulated two-phase structure of the wool keratin (Feughelman, 1959; Robson, 1966).

An inspection of Table II also shows that at a temperature of approximately 205°C the α helices should convert to the extended form in the absence of tension. This result is comparable to some of the other calculated values (Cifferi, 1963; Speakman, 1926), 140–180°C. A result obtained from X-ray measurements was about 130°C (Alexander and Hudson, 1954). A recent report for this transition temperature, obtained by heating in vacuum, is 200–225°C (Bendit, 1966). Although it is difficult to draw exact quantitative conclusions from the X-ray data, it appears that the proposed model is capable of explaining the intensity changes observed upon stretching the fiber. In particular, the decrease in the intensity of the 5.1A meridional reflection correlates reasonably well with the predicted decrease of the α helix content at a given tension. In addition, the proposed model implies that the characteristic wide-angle β reflection should occur at relatively small extensions in agreement with experiment (Bendit, 1957, 1960).

In summary, the present work strongly supports the idea [which has been questioned as a result of Bendit's (1957, 1960) quantitative X-ray studies of the stretching process] that the reversible $\alpha \rightleftarrows \beta$ transformation in the yield region is in fact explainable on the basis of a molecular unfolding process which is primarily energetic in nature.

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