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On the Ultrastructure of Cellulose Microfibrils

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Synopsis

Statistical formulae based on generally accepted models for cellulose microfibril structure were developed to calculate the mean length of microfibril segments which do not contain molecular chain-ends, i.e., "continuous segments." The calculated length of such "continuous segments" for microfibrils of 2–3 mμ cross section, containing molecules of a degree of polymerization from 2×10^3 to 6×10^3, is in the range of 300–1500 A. The relationship between "continuous segments" and crystalline and amorphous regions of cellulose microfibrils is discussed.

Introduction

The width of bacterial cellulose microfibrils (elementary fibrils in the nomenclature of Frey-Wyssling and Mühlethaler[1–3]) was considered until recently to be about 70–300 A.[4–7] Similar dimensions were attributed to cellulose microfibrils from animal[8] and plant sources.[1,9,10]

Using an improved method[11] for the measurement of the lateral dimensions of metal-shadowed bacterial and plant cellulose microfibrils, we were able to show that the above figures are an overestimation, the width of the microfibril being only about 30–35 A. and its thickness about 16–20 A.[12–15] Similar results were obtained for the width of plant microfibrils[2,14,15] and bacterial cellulose[3,14,15] by use of negative staining technique. Thus, it appears to be well established that the cellulose microfibrils from many different sources have a rectangular cross section of about 2–3 mμ.

The arrangement of cellulose molecules inside the microfibril was largely discussed by many authors.[1–3,9,10,16–18] It is now generally accepted that cellulose molecular chains are parallel to the long axis of the microfibril. Adjacent chains overlap over segments of varying length. The lateral order is variable, highly ordered regions being considered as crystalline and less ordered regions as amorphous. The length of crystalline regions is smaller than the length of a single molecule. Consequently, a molecule can traverse regions of alternating crystallinity. The amorphous region is by far more sensitive to acid degradation than the crystalline one.[16,19–21] On the basis of x-ray studies,[22] differential hydrolysis, and electron microscopy,[9,18–20,23] it was concluded that the length of the crystallites is about

600 A. (about 120 monomers) or more. Their width (uncorrected for metal deposited by shadow casting) was estimated from measurements of electron micrographs to be 50–70 A.[19,20]

However, Morehead[19] and Heyn[24,25] have found also crystallites 22–42 A. wide, a value more compatible with the lateral dimensions of cellulose microfibrils as established recently.[2,3,12–15] Similar low values were calculated from x-ray diffraction patterns.[26]

It has been assumed that crystalline regions in the microfibril are surrounded by or embedded within the amorphous material. Since the lateral dimensions of the microfibril are found to contain only about 16–36 molecules in cross section, lateral alternation of crystalline and amorphous material seems unlikely. It appears thus probable that amorphous and crystalline regions alternate only along the length of the fiber. It is also possible that amorphous regions coincide with microfibril regions containing molecular chain-ends.

The aim of this work has been to find out whether the distribution of the molecular chain-ends along a microfibril is related to the crystallite length. For this purpose the mean length of a microfibril region which does not contain chain-ends (which will be referred to as a "continuous segment") has to be calculated. This would be possible with the aid of several assumptions concerning the internal arrangement of cellulose molecules, their number in cross section, and their length.

Results and Discussion

The number of molecules (m) in the cross section of a microfibril can be deduced from its lateral dimensions. In the following calculations we shall use data for m based on lateral dimensions as previously accepted (100–300 A. range) as well as those established by more appropriate techniques (16–35 A. range).[2,3,14,15]

The length of cellulose molecules expressed in number of monomers N, was found by many authors to be in the range of 2,500–10,000.[6,7,27–30] The N values for a given sample show a normal distribution which often has a very narrow range.[28] In the model used below as a basis for our calculations we shall assume that N has a definite constant value.

We further assume that: (*1*) molecular endpoints are distributed randomly along the microfibril; (*2*) two parallel chains can be displaced longitudinally toward each other only by a whole monomer unit length (this assumption is based on the general structural feature of the crystallographic unit cell of cellulose microfibrils[31]); (*3*) there are no gaps between the ends of two consecutive chains situated on the same line along the long axis of the microfibril. The above assumptions concern only an "ideal microfibril" model. Departures from this model will be considered as well.

Based on this model, Mejzler[32] has worked out a formula (1) which enables us to calculate the mean length of a continuous segment as defined above.

Let N be the degree of polymerization of the cellulose molecule, m the number of molecules in the cross section of a microfibril, $P(n,m)$, the relative frequency of a "continuous segment" of length n, and $F(n,m)$, the overall relative weight of the fragments of length n. If N has a large value it is possible to show that the distributions $P(n,m)$ and $F(n,m)$ may be calculated to a close approximation by using the equations:

$$P(n,m) = \beta(1 - \beta)^{n-1} \tag{1}$$

$$F(n,m) = n\beta^2(1 - \beta)^{n-1} \tag{2}$$

where

$$\beta = 1 - e^{-m/N} \tag{3}$$

The mean value of $\bar{n}$ for the distribution of P, and $\bar{n}_w$ for the distribution of F can be calculated by using eqs. (4) and (5):

$$\bar{n} = 1/\beta \tag{4}$$

$$\bar{n}_w = (2 - \beta)/\beta \tag{5}$$

If m/N is a small number, then $\beta = m/N$, and hence $\bar{n}$ and $\bar{n}_w$ can be calculated by more simplified formulae:

$$\bar{n} \doteq N/m \tag{6}$$

$$\bar{n}_w \doteq 2N/m \tag{7}$$

The values $\bar{n}$ and $\bar{n}_w$ for different values of m and N were calculated by means of eqs. (6) and (7); the results are given in Table I.

The structure of cellulose microfibrils may differ in many respects from the model used as the basis for these calculations and we shall now consider to what extent deviations from this model affect the calculated values.

(*1*) Cellulose samples are polydisperse with regard to the value of N. The relationship between $\bar{n}$ and N for different values of m can be calculated from eq. (4) and is represented in Figure 1. (*2*) It is not known whether gaps between the ends of two molecules situated on the same long axial

TABLE I

Calculated Values for $\bar{n}$ and $\bar{n}_w$ for Different Values of m and N[a]

	N = 2500		N = 6000	
m	$\bar{n}$	$\bar{n}_w$	$\bar{n}$	$\bar{n}_w$
16	157	313	375	749
36	69.9	139	167	333
300	8.8	16.7	20.5	40
1000	3.0	5.0	6.5	20

[a] N = degree of polymerization. m = number of molecules in cross section of the microfibril. The values of m and N are taken from the literature,[6-8,13,19,27,28] m being calculated on the basis of lateral dimensions of the microfibril and the cellulose crystallographic unit cell.[31]

line lead to discontinuities, nor what are the lengths of such discontinuities and their distribution. Data obtained so far by means of the negative staining technique[2,3,14,15] support the view that if such discontinuities exist, they are less than about 30 × 30 × 30 A. Considering the rigidity and tensile strength properties of cellulose microfibrils[3,18] and the small number of molecules they contain in cross section, it is conceivable that discontinuities are probably few, not extensive, and therefore can be neglected in the above calculations.

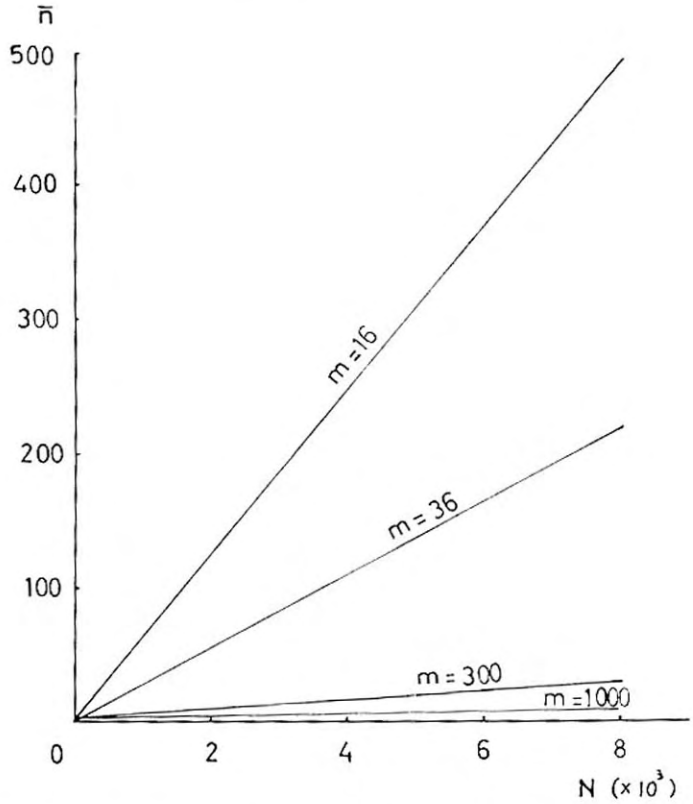

Fig. 1. Relationship between the calculated mean length of a "continuous segment" n and the degree of polymerization N for microfibrils containing different numbers of molecules in cross section m.

The data presented in Table II show good correlation between the length of a "continuous segment" and the crystallites of cellulose microfibrils when the number of molecules in the cross section is small. These results are consistent with the hypothesis that crystalline regions and "continuous segments" are identical.

It was proposed that the amorphous regions which are easily hydrolized by mild acid treatment are distributed randomly along the microfibril length.[21] This would be readily explainable assuming that the hydrolytic cleavage of cellulose microfibrils starts at amorphous regions around randomly distributed chain-ends, since in a microfibril of 2–3 mμ cross section, nearly all the molecules, and therefore nearly all the chain-ends, are on the surface of the microfibril and exposed to hydrolysis.[3,13] Thus the possible identity between crystallites and "continuous segments" as de-

TABLE II
Calculated Length of Continuous Segments ($\bar{n}$) for Microfibrils of Different Lateral Dimensions as Compared with Experimental Data for Crystallite Length

Cross section, A^2[a]	% of molecules on the surface of microfibril[a]	m[a]	References	$\bar{n}$[b]	Crystallite length[c]	References
5×10^2	75–100	16	13	150–375	100	18,20,23
					110–280	33
1.2×10^3	60	36	3	69–167	150–450	26
					60–200	34
10^4	25	300	4–8	8–20		
9×10^4	15	1000	1,10	3–6		

[a] Calculated values from the lateral dimensions of microfibrils as determined from electron micrographs and the crystallographic unit cell of cellulose.[31]

[b] The lower values of $\bar{n}$ are calculated for $N = 2500$; the higher value for $N = 6000$ (Table I).

[c] Length is expressed as degree of polymerization.

TABLE III
Calculated Weight Per Cent p Removed by the Dissolution of a Microfibril Segment of Length a for Different Values of m and N[a]

		p					
m	N	$a = 10$	$a = 20$	$a = 30$	$a = 40$	$a = 50$	$a = 60$
16	2500	6.16	11.9	17.4	22.5	—	—
	6000	2.61	5.2	7.7	10.0	12.4	14.7
36	2500	13.2	24.7	—	—	—	—
	6000	5.8	11.2	16.4	21.2	—	—
300	2500	65.5	—	—	—	—	—
	6000	37.2	—	—	—	—	—
1000	2500	95.2	—	—	—	—	—
	6000	75.9	—	—	—	—	—

[a] a and N are expressed in monomer units.

scribed above provides a further basis for the understanding of the initial hydrolysis of cellulose microfibrils.

Assuming that the hydrolysis occurs only at amorphous regions around molecular chain-ends, it is also possible, by using eq. (8), to calculate the weight per cent (p) of cellulose removed after the dissolution of a segment of length a (—monomer units).

$$p = 1 - e^{-am/N}[a(e^{m/N} + e^{-m/N}) - (2a - 1)] \qquad (8)$$

Table III shows the results of this calculation.

Résumé

Des formules statistiques, basées sur les modèles généralement acceptés concernant la structure des microfibrilles de cellulose, ont été développées afin de calculer la longueur moyenne des segments microfibrillaires ne contenant pas de bouts de chaînes moléculaires. La longueur calculée de ces segments pour des microfibrilles d'une section transversale de 2 à 3 mμ et contenant des chainons cellulosiques d'un degré de polymérisation de 2 à 6 $\times$ 10^3, se situe entre 300 et 1500 A. Les relations possibles entre ces segments, et les régions cristallines et amorphes des microfibrilles de la cellulose sont envisagées.

Zusammenfassung

Es wurden statistische, auf einem allgemein angenommenen Modell für die Struktur von Zellulosemikrofibrillen beruhenden Formeln entwickelt, um die Länge von Segmenten, die keine Endketten enthalten, zu berechnen. Die ungefähre Länge solcher durchlaufender Ketten von Mikrofibrillen mit etwa 2–3 mμ Durchmesser, welche Moleküle vom Polymerisationsgrad 2–6 $\times$ 10^3 enthalten, wurde zu 300 bis 1500 A. berechnet. Die Beziehungen zwischen durchlaufenden Segmenten und kristallinen und amorphen Bereichen von Zellulosemikrofibrillen werden besprochen.

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