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SINGLE CRYSTALS OF DEXTRAN: HIGH TEMPERATURE POLYMORPH

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ABSTRACT

Lamellar single crystals of a high temperature polymorph of synthetic dextran were prepared at temperatures ranging from 120 to 200°C in a mixture of water and polyethylene glycol. Individual crystals with lath-like shapes gave well resolved electron diffraction diagrams from which the reciprocal unit cell parameters a^*, b^* and γ^* could be measured. The direct cell parameters were then determined from a series of electron diffraction diagrams obtained by sequential tilting of the crystal about the b^* axis. This gave $a = 0{\cdot}922 \pm 0{\cdot}001$ nm, $b = 0{\cdot}922 \pm 0{\cdot}001$ nm, c (chain axis) $= 0{\cdot}78 \pm 0{\cdot}01$ nm, $\alpha = \gamma = 90°$ and $\beta = 91{\cdot}3° \pm 0{\cdot}5°$. The crystal symmetry was $P2_1$ with b as the unique monoclinic axis. These data coupled with the observed density of the crystals, indicated that the unit cell contained two antiparallel dextran chains of two residues each. When the crystals were grown at temperatures between 90 and 120°C, a percentage of crystals containing both low and high temperature polymorphs were obtained. These mixed crystals had most likely grown in syntaxy.

1. INTRODUCTION

Polymorphism is a phenomenon frequently observed in the crystallisation of polysaccharides, especially in their underivatised form. This occurs because a polysaccharide chain, in a given conformation, has several ways to associate with its neighbours through hydrogen bonds. An example of this type of polymorphism is the case of cellulose for which at least four different polymorphs have been described, differing only in crystal packing but not in chain conformation (Sarko, 1976, 1978). Another type of polymorphism, also encountered in polysaccharides, involves a change in chain conformation in addition to that in chain packing. This latter case is

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classically illustrated by amylose, for which a series of crystal structures exist, each with a different chain conformation (Marchessault & Sarko, 1967; French & Murphy, 1977).

Among the parameters that are critical for the control of polymorphism in polysaccharides, the temperature of crystallisation is the most important. As a consequence, high temperature and low temperature polymorphs have been found for several crystalline polysaccharides such as cellulose, curdlan, etc. (Wellard, 1959; Marchessault *et al.*, 1977). The crystallisation medium is also important as quite frequently a change in solvent will yield a different polymorph, this being again illustrated by the crystallisation of amylose (Marchessault & Sarko, 1967; French & Murphy, 1977). Finally, one must mention the spectacular changes that sometimes occur when native crystalline polysaccharide material is recrystallised from solution, as for instance in cellulose.

Dextran with its $\alpha(1 \rightarrow 6)$ backbone linkage is a suitable candidate for polymorphism, due both to the flexibility of the chain as well as the multiple potential for intramolecular hydrogen bonds within the crystals. The classical work of Jeanes *et al.* (1948) clearly showed this potential. However, because well oriented X-ray diffraction diagrams of dextran have not been obtained, the polymorphism of dextran has not been well described so far.

In a previous paper (Chanzy *et al.*, 1980), we reported the preparation and crystallographic data of single crystals of dextran prepared at temperatures between room temperature and 90°C. The present work describes another polymorph of dextran also giving single crystals, but at higher temperatures. These crystals yield high quality diffraction information which may be suitable for a three-dimensional crystal structure determination.

2. EXPERIMENTAL

2.1. *Materials*

The synthetic dextran used in this work was prepared according to the procedure of Schuerch & Uryu (1972), with the experimental conditions as described previously (Chanzy *et al.*, 1980). The sample utilised for the crystallisation experiments had a degree of polymerisation (DP) of 60, 2% of residual $\beta(1 \rightarrow 6)$ linkages as determined by C^{13} NMR spectroscopy (courtesy of D. M. Vignon using a 250 MHz Cameca spectrometer), and an optical rotation $[\alpha]_D^{25}$ of + 95°.

2.2. *Crystallisation*

The crystallisation experiments were achieved at temperatures between 120 and 200°C in the apparatus shown in Fig. 1. This consisted of a series of vessels that allowed the precipitation of dextran from its aqueous solution by the addition of polyethylene glycol (PEG) at the crystallisation temperature. The procedure was as

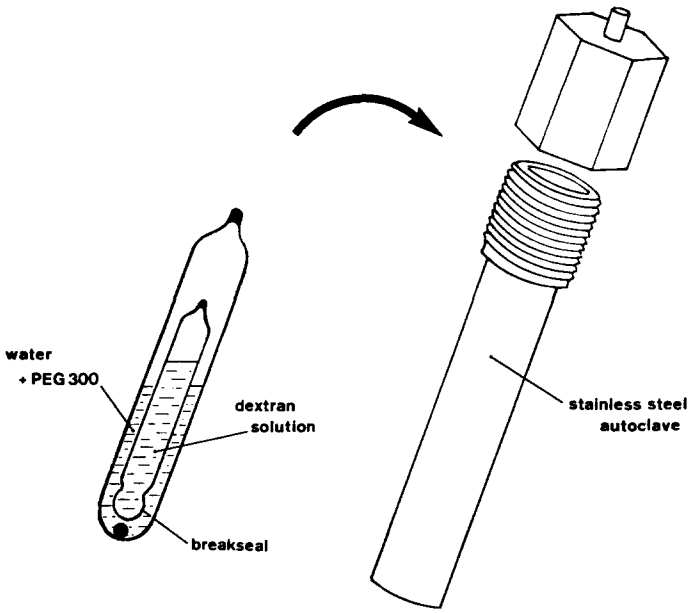

Fig. 1. Crystallisation apparatus for the preparation of high temperature synthetic dextran single crystals.

follows: A 0·05% dextran solution in water was stabilised against spontaneous crystallisation by heating it at 180°C for 1 h in a pressure vessel. The solution was cooled and filtered through a low porosity, fritted glass filter. The dextran solution was then poured into an ampoule fitted with a breakseal. The ampoule was sealed and positioned inside a glass container holding a mixture of water and PEG 300 (from Fluka, freshly purified by high speed centrifugation). The composition of the PEG/water mixture was chosen so that the final crystallisation conditions of dextran were achieved in mixtures containing from 60 to 80% (v/v) PEG 300. The glass container was also sealed and placed inside a stainless steel autoclave which was immersed in a constant temperature oil bath. When the crystallisation temperature was reached, the autoclave was shaken in order to break the breakseal, resulting in the mixing of the dextran solution and the precipitating agent. Crystallisation usually occurred within 1 h at the preset temperatures. For the highest temperatures, however, shorter times of crystallisation were used in order to avoid degrading the dextran.

2.3. *Electron Microscopy*

The dextran crystals were collected and washed by successive centrifugations in methanol. A drop of the crystal suspension was then deposited on a carbon-coated

grid. The crystals were observed with Philips EM 300 and Philips EM 400 T microscopes. Imaging was achieved at 80 kV on preparations shadowed with W/Ta alloy. Electron diffraction diagrams were recorded at 120 kV on tilted and untilted crystals, using a rotation specimen holder. Tilt angles between $-60°$ and $+60°$ were used. The electron diffraction diagrams were calibrated with gold.

2.4. *X-ray Diffraction*

Powder X-ray diagrams were recorded with a Warhus flat-film vacuum camera, using Cu Kα radiation. The patterns were calibrated with calcite.

3. RESULTS AND DISCUSSIONS

Compared with the preparation of the low temperature dextran polymorph, the crystals of the high temperature polymorph were readily obtained. No serious nucleation problems were encountered. The property of PEG to precipitate and crystallise dextran was verified, in agreement with the observation of Kochetkov *et al.* (1977).

A typical batch of crystals is shown in Fig. 2. The sample, prepared at 160°C, consists of a large number of lath-like, lamellar crystals several microns in length and approximately 1 μ or less in width. As a rule, the lamellae radiate from a thick nucleation centre, which results in frequent overlapping of the crystals in the form of stacks. The thickness of the individual lamellae was estimated at 7 nm, a value that compares well with the thickness normally found in polymer single crystals.

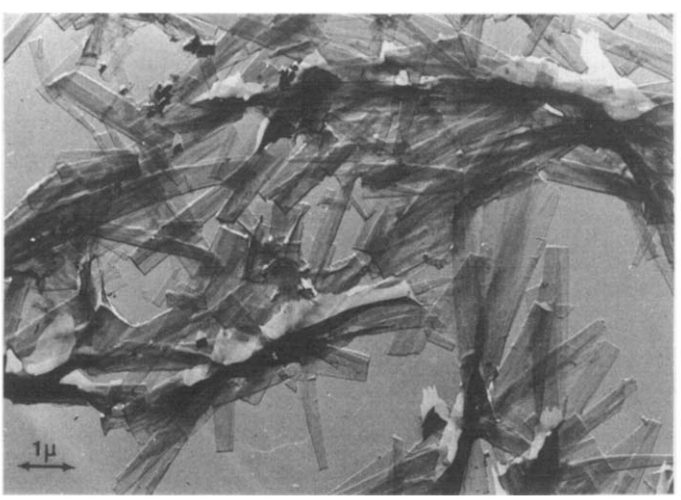

Fig. 2. Single crystals of synthetic dextran obtained at 160°C in a solution containing 35% water and 65% (v/v) PEG. Shadow cast with W/Ta alloy.

Individual dextran crystals gave good quality electron diffraction diagrams. This is illustrated in Fig. 3 where the diffraction diagram shown in B is in proper orientation relative to the single crystal shown in A. The diagram displays diffraction spots located at the intersection of a nearly square grid. A study of the diffraction intensities reveals that the diagram possesses two perpendicular axes of symmetry a^* and b^*, with b^* being aligned with the length of the crystal. All of the information is thus contained in one quadrant of the diagram. Although a^* and b^* have identical lengths, they are symmetrically not equivalent since on b^* only even reflections are present, whereas no such systematic absences could be detected along a^*. For this reason, four-fold symmetry has to be ruled out. As shown in Fig. 3B, the diagrams are well resolved, containing 50 independent measurable reflections, yielding information down to 0·1 nm. This denotes a rather high perfection of the molecular packing within the crystals.

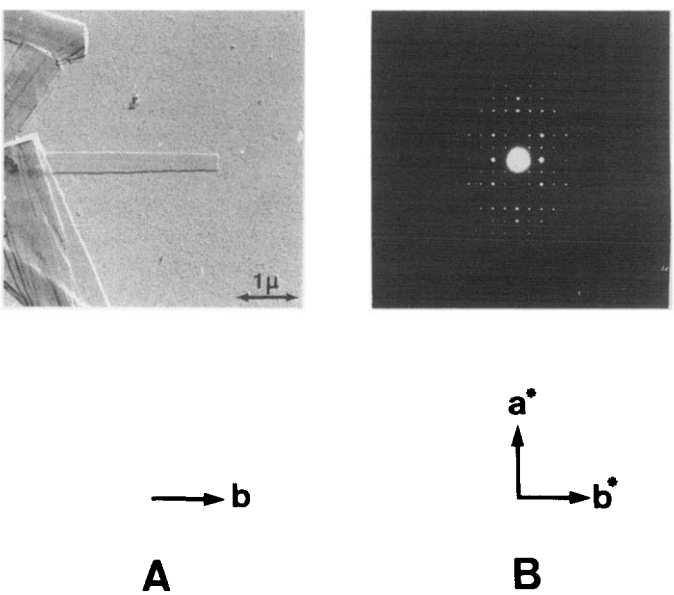

Fig. 3. A, Single crystals of synthetic dextran obtained as in Fig. 2. B, Corresponding electron diffraction diagram properly oriented with respect to the crystals. The axes of the diagram are indicated.

The parameters of the direct unit cell were determined from a sequence of diagrams obtained by tilting around b^*. The results are shown in Fig. 4(A-E). Each electron diffraction diagram of a tilted crystal again displays two perpendicular axes of symmetry around which the diffraction intensities are distributed in four equivalent quadrants. However, the diagrams obtained from a given crystal rotated clockwise

show different intensities compared with the diagrams obtained from the same crystal in a counterclockwise tilting of the same magnitude. This is well illustrated in Fig. 4B-E, corresponding to + (clockwise) and − (anticlockwise) 31° and 50° tilts. All these observations lead to the following general conditions:

$$|F(hkl)| = |F(\bar{h}\bar{k}\bar{l})| = |F(h\bar{k}l)| \neq |F(\bar{h}kl)|$$

$$|F(oko)| = 0 \text{ when } k = 2n + 1$$

These conditions indicate without ambiguity that we are dealing with the $P2_1$ space group with b as the unique axis. A calibration of the tilted and untilted diagrams leads to the unit cell parameters $a = 0{\cdot}922 \pm 0{\cdot}001$ nm, $b = 0{\cdot}922 \pm 0{\cdot}001$ nm, c (chain axis) $= 0{\cdot}78 \pm 0{\cdot}01$ nm, $\alpha = \gamma = 90°$ and $\beta = 91{\cdot}3° \pm 0{\cdot}5°$. As shown in Table 1, the electron diffraction data are in good agreement with the X-ray measurements obtained from powder samples of dextran.

From the unit cell and symmetry data, the probable number of chains in the cell can be determined as follows. Since the crystals have a measured density of 1·60 g/cc, the number of glucose residues per unit cell is 4, assuming that no water is present in the cell. When the 2_1 symmetry along b is accounted for, this gives a model of two dextran chains with two residues each, the two chains being antiparallel as required by the screw axis. Furthermore, the dextran chains are perpendicular to the plane of the lamellar crystals, a common behaviour for polymer single crystals (Geil, 1963). Also, since many single crystals are monolamellar, approximately 7 nm in thickness, it is likely that the chains are folded within the crystal. This behaviour, which is observed in crystals of flexible polymer molecules (Geil, 1963), accords with the flexibility of the dextran chain.

The crystallographic and symmetry parameters determined above are rather interesting in the sense that the 2_1 axis of the unit cell does not coincide with the chain direction but is perpendicular to it. This occurrence, although not common in polymer crystals, has previously been observed in other polysaccharide crystals (Sarko & Zugenmaier, 1980). In the latter cases, however, it has not been possible to determine the precise value of the monoclinic angle when it was close to 90° and the data were obtained by X-ray diffraction. The reason for this is that an X-ray fibre diagram has insufficient resolution to determine the intensities of the overlapped (hkl) and $(\bar{h}kl)$ diffractions. In this instance, the electron diffraction technique is far superior. It remains to be seen whether the electron diffraction intensities can be used for the complete structure determination, and the structure of the high temperature polymorph of dextran is currently being refined, using only the electron diffraction intensities. The results of this investigation will be published in due course (Guizard *et al.*).

The diffraction data on dextran obtained here can be compared with the X-ray data on dextran already in the literature (Jeanes *et al.*, 1948; Rückel & Schuerch, 1966; Kobayashi, 1968; Barham *et al.*, 1974; Kiselev *et al.*, 1976; Kochetkov *et al.*, 1977; Stipanovic, 1978). The patterns of the crystals presented above contain one

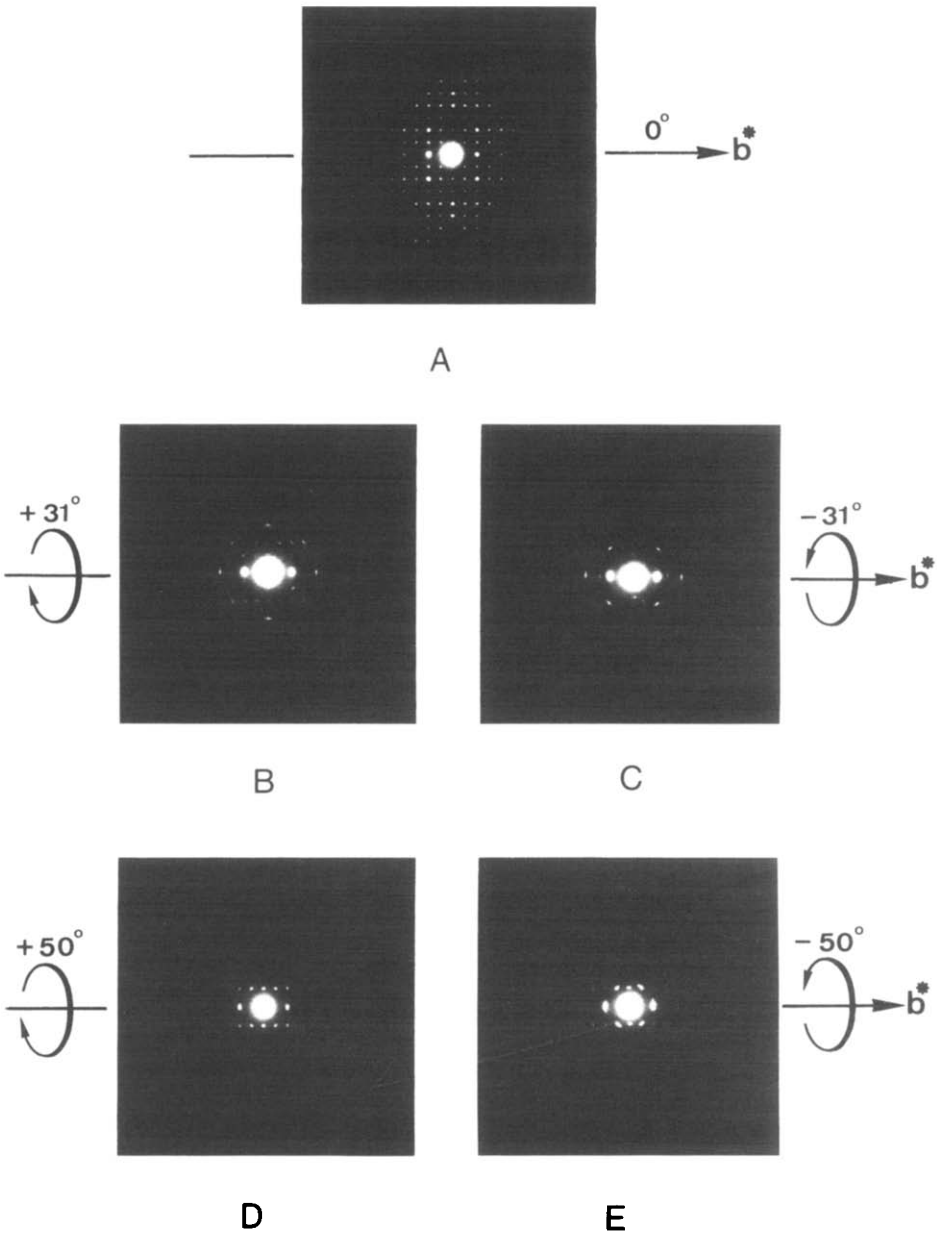

Fig. 4. Electron diffraction diagrams obtained by sequential tilting around b^* of a dextran crystal prepared as in Fig. 2. A, Zero tilt; B, +31° tilt; C, −31° tilt; D, +50° tilt; E, −50° tilt.

TABLE 1

Interplanar *d* Spacings from Electron Diffraction and X-ray Powder Diagrams of Dextran. The Indexing was Obtained with the Unit Cell Parameters Deduced from Electron Diffraction Patterns ($a = 0{\cdot}922$ nm, $b = 0{\cdot}922$ mn, $c = 0{\cdot}778$ nm, $\alpha = \gamma = 90°$, $\beta = 91{\cdot}3°$)

h	*k*	*l*	*Calculated d spacing (nm)*	*Observed d spacing (nm) from electron diffraction data*	*Observed d spacing (nm) from X-ray data*	*Visual intensity from X-ray data*[a]
1	0	0	0·922	0·922	–	–
0	1	0	0·922	0·922	–	–
$\bar{1}$	1	0	0·652	0·652	0·651	s
$\bar{1}$	0	1	0·602	0·596	0·595	m (broad)
$\bar{1}$	0	1	0·588	0·582		
$\bar{1}$	1	1	0·504	0·505	0·501	s (broad)
1	1	1	0·496	0·496		
2	0	0	0·461	0·461	0·461	vs
0	2	0	0·461	0·461		
2	$\bar{1}$	0	0·412	0·412	0·411	m
$\bar{1}$	2	0	0·412	0·412		
$\bar{2}$	0	1	0·401	0·399	0·391	m (broad)
0	2	1	0·396	0·397		
$\bar{2}$	0	1	0·393	0·389		
$\bar{2}$	1	1	0·367	0·368	0·364	m
$\bar{1}$	2	1	0·366	0·366		
1	2	1	0·363	0·363		
2	1	1	0·361	0·361		
2	2	0	0·326	0·326	0·325	s
$\bar{3}$	0	0	0·307	0·307	0·303	w (broad)
$\bar{2}$	2	1	0·302	0·302		
$\bar{2}$	0	2	0·301	0·301		
2	2	1	0·299	0·298	0·292	w (broad)
0	2	2	0·297	0·298		
3	1	0	0·291	0·291		
1	3	0	0·291	0·291		
$\bar{1}$	2	2	0·281	0·283	0·280	vw (broad)
$\bar{1}$	3	1	0·274	0·273		
3	2	0	0·256	0·256	0·256	vw
$\bar{2}$	3	0	0·256	0·256		
$\bar{2}$	3	1	0·244	0·243	0·242	vw
2	3	1	0·242	0·241		
3	0	2	0·238	0·238	–	–
4	0	0	0·230	0·230	0·228	vw
0	4	0	0·230	0·230		
4	1	0	0·224	0·224	0·222	vw
1	4	0	0·224	0·224		
3	3	0	0·217	0·217	–	–
4	2	0	0·206	0·206	0·204	vw
2	4	0	0·206	0·206		
$\bar{4}$	0	2	0·196	0·196	0·193	vw
$\bar{1}$	3	3	0·194	0·195		
1	3	3	0·193	0·193		
5	0	0	0·184	0·184	0·185	vw
4	3	0	0·184	0·184		
3	4	0	0·184	0·184		
5	1	0	0·181	0·181	–	–
1	5	0	0·181	0·181		
5	2	0	0·171	0·171	0·170	vw
2	5	0	0·171	0·171		
5	3	0	0·158	0·158	–	–
3	5	0	0·158	0·158		
6	0	0	0·154	0·154	–	–
0	6	0	0·154	0·154		
6	1	0	0·152	0·152		

Table 1–*Continued*

h	*k*	*l*	*Calculated d spacing (nm)*	*Observed d spacing (nm) from electron diffraction data*	*Observed d spacing (nm) from X-ray data*	*Visual intensity from X-ray data*[a]
1	6	0	0·152	0·152	–	–
6	2	0	0·146	0·146		
2	6	0	0·146	0·146	–	–
5	0	3	0·147	0·147	–	–
5	4	0	0·144	0·144	–	–
4	5	0	0·144	0·144		
6	3	0	0·137	0·137	–	–
3	6	0	0·137	0·137		
1	7	0	0·130	0·130		
5	5	0	0·130	0·130	–	–
6	4	0	0·128	0·128	–	–
7	2	0	0·127	0·127	–	–
7	3	0	0·121	0·121	–	–
6	5	0	0·118	0·118	–	–
7	4	0	0·114	0·114	–	–
7	5	0	0·107	0·107	–	–

[a] vs = Very strong, s = strong, m = medium, w = weak, vw = very weak.

very strong diffraction at 0·46 nm and three strong ones at 0·65, 0·50 and 0·32 nm. Some of these values match well with those obtained by other workers. For instance, the diagrams L2 and L3 published by Jeanes *et al.* (1948) both contain a strong or medium intensity line at 0·45 nm; this is also found in the X-ray pattern of spherulites prepared by Barham *et al.* (1974). On the other hand, our X-ray patterns are decisively different from many other published dextran patterns (Rückel & Schuerch, 1966; Kobayashi, 1968; Barham *et al.*, 1974; Kiselev *et al.*, 1976; Stipanovic, 1978) and those obtained with the lower temperatures of crystallisation (Chanzy *et al.*, 1980). This discrepancy clearly indicates the presence of a variety of dextran polymorphs. Their occurrence is in agreement with the known chain flexibility of this polymer and its multiple minimum energy conformations (Tvaroska *et al.*, 1978).

A final interesting observation was made when the dextran was crystallised at temperatures between 90 and 120°C. At these temperatures, mixed preparations containing both crystals of high and low temperature polymorphs were obtained. More interesting was the observation of a percentage of hybrid crystals, having both high and low temperature polymorphs present at the same time. This is illustrated in the crystal pictured in Fig. 5A. The crystal has a lozenge lamellar centre (the low temperature polymorph) from which four sets of lath-like lamellae, corresponding to the high temperature polymorph, radiate in a cross-like fashion. The two types of crystals are intertwined, indicating that they come from a hybrid seed which has propagated both morphologies. The four sets of high temperature crystals correspond to the four sides of the lozenges and are 54° apart. In the electron diffraction mode (Fig. 5B), the crystals in Fig. 5A give a composite electron diffraction diagram which is the superposition of a diagram of the low temperature polymorph and two diagrams of the high

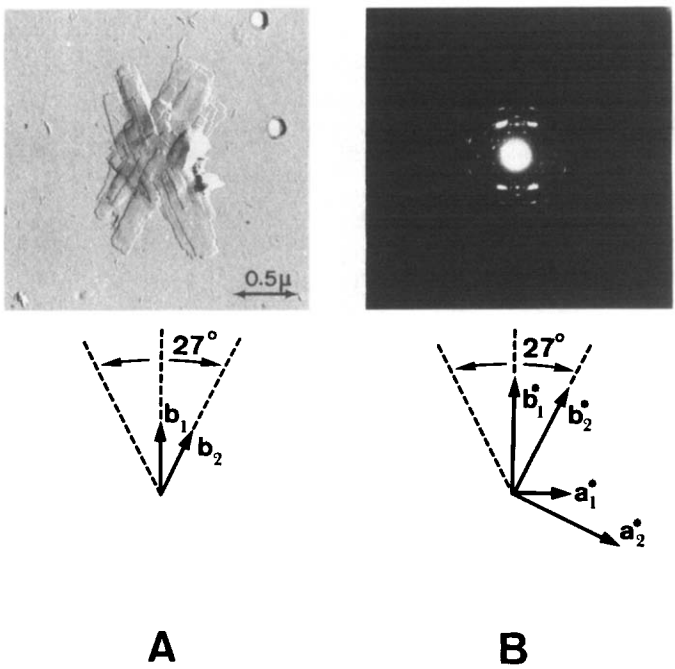

Fig. 5. A, Hybrid crystal of synthetic dextran grown at 110°C in a solution containing 50% water and 50% (v/v) PEG. B, Corresponding electron diffraction diagram properly oriented with respect to the crystal. The principal axes are indicated.

temperature polymorph. As shown in Fig. 5B, each high temperature diagram is rotated by 27° from the low temperature one and the two high temperature diagrams are 54° apart.

The observations in Fig. 5 can be compared with related observations made on cellulose where hybrid crystals could also be grown by controlling the temperature of crystallisation (Buleon & Chanzy, 1980). In the case of cellulose, it was concluded that the crystal growth had gone through a syntaxy mechanism. The two different parts of the crystal consisted of chains of identical conformation but different packing. In the present case, a similar occurrence may also be the case as both crystal structures have comparable chain axes: $c = 0{\cdot}78$ nm for the high temperature polymorph versus 0·81 nm for the low temperature polymorph. This points toward closely related conformations for the dextran chains in both cases. The polymorphism would then be explained only by different packing of the chains. It may be recalled also that the low temperature polymorph has some water present within its unit cell (Chanzy *et al.*, 1980) whereas there is none in the high temperature polymorph. This may be sufficient to change the packing of the dextran chains from one to the other polymorph. At temperatures where water is still partially associated with the dextran

molecules, the two different packing arrangements can exist at the same time, thus explaining the syntaxial growth of the crystals as in Fig. 5.

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