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Preliminary communication

The crystal structure of A-starch is it double helical?

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It has been known for a long time that granular starches are crystalline and that they exist in at least two polymorphic forms, the A- and B-starches[1] The former are usually found in cereals, whereas the latter are found in tubers A rare, third polymorph (C-starch) has been claimed to exist by some workers[1,2] While the biological necessity for crystallinity in starch granules is puzzling, its polymorphism is even more puzzling. Despite efforts dating back to the 1930's, the crystalline structure of starches is still unknown

We now present evidence for a new unit-cell of crystalline A-amylose, based on well oriented, X-ray fiber-diagrams The new cell accounts for all of the reflections seen in powder patterns of A-starch There is also a good probability that the crystal structure of A-amylose is based on double-stranded helices A double-helical model for starch was first suggested by Kainuma and French[3] for another polymorph, namely the B-starch

An X-ray fiber diagram of A-amylose is shown in Fig 1 The fiber from which this diagram was recorded was obtained by solid-state deacetylation of oriented, crystalline, amylose triacetate, using a modification of the method first used by Senti and Witnauer[4] for preparation of amylose fibers Fully acetylated amylose (potato amylose, $\bar{M}_w$ = 750,000, courtesy of Dr F R Dintzis, USDA Laboratories, Peoria, Ill) was drawn into a fiber as described by Sarko and Marchessault[5], then deacetylated in 0 2*M* potassium hydroxide (75% ethanol) for 24 h while being kept under tension The resulting "alkali amylose" was exposed to 80% relative humidity for 12 days at 85°, followed by 100% relative humidity for 6 days at 90° The X-ray diagram was recorded on flat film (Ilford Type G) with the fiber in an atmosphere of 80% relative humidity, using a Searle, toroidal focusing camera and nickel-filtered, copper K_α radiation Internal calibration with sodium fluoride was used for diagrams intended for the measurement of *d*-spacings

The diagram may be indexed with an orthorhombic unit cell a = 11 90, b = 17 70, and c (fiber repeat) = 10 52 Å The indexing is shown in Table I The space group cannot be determined, but the common space-groups $P2_1$ and $P2_12_12_1$ are ruled out A third-order

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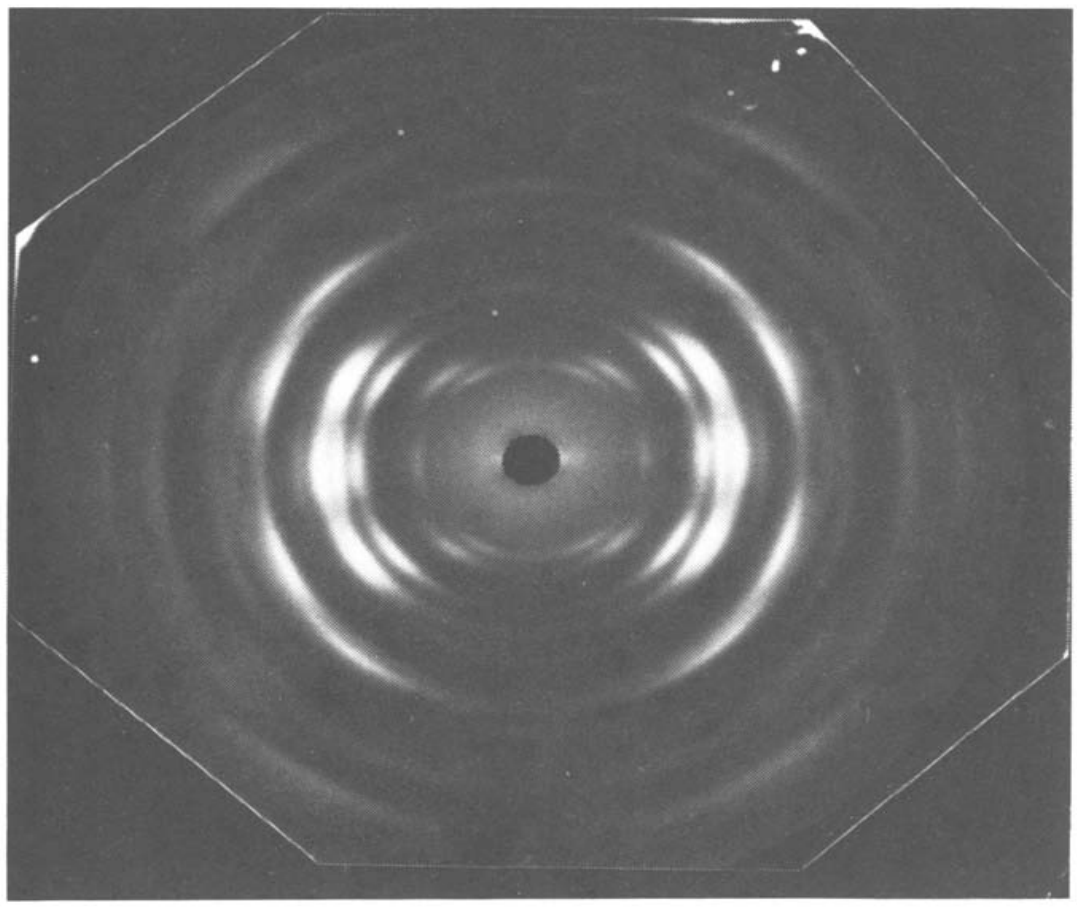

Fig 1 Fiber diagram of A-amylose The fiber axis is vertical.

meridional is clearly visible on diagrams obtained from tilted fibers All of the *d*-spacings observed in powder patterns of A-starches may be found in this diagram, however, the unit cell of A-starch proposed earlier by Bear and French[6] on the basis of powder patterns can not index the reflections of the fiber pattern

The density of the fiber, measured by flotation in benzene—chloroform, is 1 51 g/cm^3 The calculated density nearest to this figure, at 1 46 g/cm^3, is for a unit cell containing 12 D-glucose residues, which leaves room for up to eight water molecules per unit cell Results of elemental analyses performed on samples stored both at ambient and 80% relative humidity are in fair agreement with this figure, thus, a sample stored at ambient relative humidity contained 6 3 ±0 2% of water, or 7 2 ±0 3 water molecules per cell, another sample stored at 80% relative humidity contained 8 8 ±1.2% or 10 5 ±1 5 water molecules per cell

The unit-cell dimensions suggest that the 12 residues contained in this cell are equally divided among two sites, one located at the corner of the unit cell and the other at its center This arrangement is supported by Patterson projections, computed with preliminary, equatorial intensities

The presence of the third-order meridional is consistent with two possible single-helical structures and one double-helical structure The first of the single helices is a three-fold helix (either 3_1 or 3_2), and the second is a six-fold helix in which the asymmetric residue is comprised of two, non-equivalent D-glucose residues For the three-fold helix, the corner and center locations of the unit cell would have to be occupied by two chains

TABLE I

DIFFRACTION DATA FOR A-AMYLOSE

hkl	d-spacing (A)		
	Calculated	*Observed*	*Intensity*[a]
110	9 87	10 4	vvw
020	8 85	8 88	m
030	5 90	5 89	s
130	5 28	5 34	s
220	4 93	4 91	s
040	4 43	4 46	w
310	3 87	3 88	w
240	3 55	3 58	vw
150	3 39	3 39	vw
340	2 95	2 93	vw
011	9 04	9 17	m
101	7 88	7 86	m
121	5 88	5 88	s
201	5 18	5 20	s
211	4 97	4 87	s
131	4 72		
221	4 47	4 38	vvw
231	3 89	3 89	s
31ı	3 63	3 64	vvw
241	3 36	3 36	w
112	4 64	4 67	vvw
202	3 94	3 95	vw
132	3 75	3 75	s
042	3 38	3 38	vvw
312	3 12	3 11	vw
152	2 85	2 85	vw
003	3 51	3 51	w
123	3 15	3 18	vvw
203	3 02	3 00	w

[a]Intensity scale s, strong, m, medium, w, weak, v, very

each, in close proximity to one another For the six-fold helix, each site would be occupied by a single chain

The double-helical structure would consist of two helical strands, either in 6_1 or 6_5 conformation, repeating in $2c$ = 21 04 Å, with the strands wound around each other 180° apart If the strands are parallel and in phase with each other along the fiber axis, the true crystallographic-repeat would be the observed repeat of 10 52 Å If the parallel strands are slightly out of phase or the strands are antiparallel, the true repeat would be 21 04 Å, but for some such structures, odd-order layer lines would be too weak to be visible, thus giving the appearance of a repeat of 10 52 Å

The three possibilities were examined by combined conformational and packing

calculations, the details of which will be reported later Reasonable three-fold helices are impossible to construct, assuming a $^4C_1$ conformation of the D-glucose residue (The same conclusion was reached both by us[7] and by French and Murphy[8] in earlier analyses of possible chain-conformations of B-amylose, for which the fiber repeat is essentially the same as for A-amylose It is also impossible to pack four of any of the three-fold helices into the unit cell On the other hand, the six-fold single helices 6_1 and 6_5 are both conformationally probable, as has already been shown in ϕ,ψ conformational calculations performed for B-amylose[7] However, it is impossible to pack two such helices into the A-amylose unit-cell because of numerous short, nonbonded contacts between the corner and center chains Consequently, the only alternative that remains is the double helix of either parallel or antiparallel strands Conformational calculations show that it is possible to construct such helices by using the standard $^4C_1$ (D) residue conformation and residue geometry, which is in agreement with crystallographically determined structures of simple sugars The glycosidic bridge-angle for these conformations ranges from 105 to 119° Both parallel and antiparallel stranded, double helices are possible, as are right-handed and left-handed conformations Packing analysis of double helices shows a comfortable packing of chains at corner and center locations of the unit cell, with no serious short-contacts between helices There is adequate space to place water molecules between the helices, with less likelihood of placing water inside the helices, as is true for V-amylose[9,10] Preliminary difference-Fourier syntheses also indicate good agreement of such a packing arrangement with the X-ray data

It is thus clear that a double-helical, crystalline arrangement of amylose chains is a very likely structure for A-amylose and A-starch Attempts to refine it against X-ray data are in progress, and the results will be reported in due course

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REFERENCES

1 J A Radley, *Starch and Its Derivatives*, 4th edn , Chapman and Hall, London, 1968, p 153
2 S Hizukuri *Agr Biol Chem* , 25 (1961) 45–49
3 K Kainuma and D French, *Biopolymers*, 11 (1972) 2241–2250
4 F R Senti and L P Witnauer, *J Am. Chem Soc* , 68 (1946) 2407–2408
5 A Sarko and R H Marchessault, *J Am. Chem Soc* , 89 (1967) 6454–6462
6 R S Bear and D French, *J Am Chem Soc* , 63 (1941) 2298–2305
7 J Blackwell, A Sarko, and R H Marchessault, *J Mol Biol* , 42 (1969) 379–383
8 A D French and V G Murphy, *Carbohydr Res* , 27 (1973) 391–406
9 W T Winter and A Sarko, *Biopolymers*, 13 (1974) 1447–1460
10 P Zugenmaier and A Sarko, *Biopolymers* 15 (1976) 2121–2136