Carbohydrate Research, 89 (1981) 1-10
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

DETAILED STRUCTURE OF THE V_h-AMYLOSE–IODINE COMPLEX A LINEAR POLYIODINE CHAIN*

TERRY L BLUHM† AND PETER ZUGENMAIER††
Institut für Makromolekulare Chemie der Universitat Freiburg, D-7800 Freiburg i Br (German Federal Republic)

(Received September 11th, 1979, accepted for publication, June 2nd, 1980)

ABSTRACT

The structure of the hydrated amylose–iodine complex has been determined by combined methods of X-ray diffraction and stereochemical packing analysis The unit cell was identified as orthorhombic with a = 13 60 Å, b = 23 42 Å, and c = 8 17 Å (fibre repeat) with two amylose chains passing through the unit cell Iodide was found as an almost linear chain in the centre of the six-fold, left-handed amylose helix Eight water molecules of hydration per unit cell were located in good hydrogen-bonding positions between the amylose helices

INTRODUCTION

The structure of the blue starch–iodine complex has been a topic of considerable discussion[1-14] The studies by Rundle and co-workers[1 5 6] of various amyloses and amylose complexes led to the proposal that the amylose chains crystallise in a six-fold helical conformation The proposal[6], based on X-ray diffraction experiments, that iodine occupies the otherwise open channel in the centre of the amylose helix verified chemical predictions of other workers[12 13] However, a detailed structural investigation was not possible and, subsequently, the stereochemical disposition of the iodine in the central cavity of the helix was not determined West[9 10] suggested that the iodide in the amylose–iodine complex, as well as in other polymeric materials, existed as a linear array at equal interatomic distances of 3 1 Å Other investigators have presented supporting evidence[8 13] On the other hand, Stein and Rundle[7] developed a theory explaining the interaction of iodide with the amylose helix as a dipole–dipole interaction which predicts non-equivalent I I separation distances

Crystallographic studies of model compounds[15 16] of the amylose–iodine complex, such as cyclohexa-amylose complexed with iodine, have been undertaken

---

*Conformation and Packing Analysis of Polysaccharides and Derivatives, Part VII For Part VI see ref 18

†Present address Xerox Research Centre of Canada, 2480 Dunwin Drive, Mississauga, Ontario L5L 1J9, Canada

††Present address Institut fur Physikalische Chemie der T U Clausthal, 3392 Clausthal-Zellerfeld, German Federal Republic

In these studies, the complexed iodine was present as either I_2, I_3^-, or I_5^- species. However, these model compounds do not exhibit the continuous nature of the amylose helix. In a recent study[27] with the resonance Raman/iodine Mossbauer technique, I_5^- was detected in the iodine–starch complex.

In view of recent successes in solving polysaccharide structures involving complexation with small molecules[17,18], we have reinvestigated the amylose–iodine complex. In this investigation, X-ray diffraction methods were combined with those of stereochemical packing analysis[19]. Debye–Scherrer X-ray intensity data[20], as well as X-ray data from amylose–iodine fibre diagrams, were used.

EXPERIMENTAL

V-Amylose–iodine films were prepared by casting films from a 15% (w/w) solution in Me_2SO. The films were then stretched in high relative humidity at room temperature to an extension of 200–600%. These stretched films, which produced oriented-fibre X-ray diagrams typical of the Me_2SO–amylose complex[21], were then placed in boiling methanol for 20 min whilst held under tension, after which they exhibited X-ray diagrams of V_a-amylose. The films were next placed, still under tension in a chamber containing iodine vapour at 100% relative humidity for 6 h. After this treatment, the films possessed the intense blue colour characteristic of the amylose–iodine complex and gave the fibre X-ray diagram shown in Fig. 1.

X-Ray diffractograms for *d*-spacing measurements were recorded on flat film in an evacuated camera at a film-to-sample distance of 5 cm. For intensity measure-

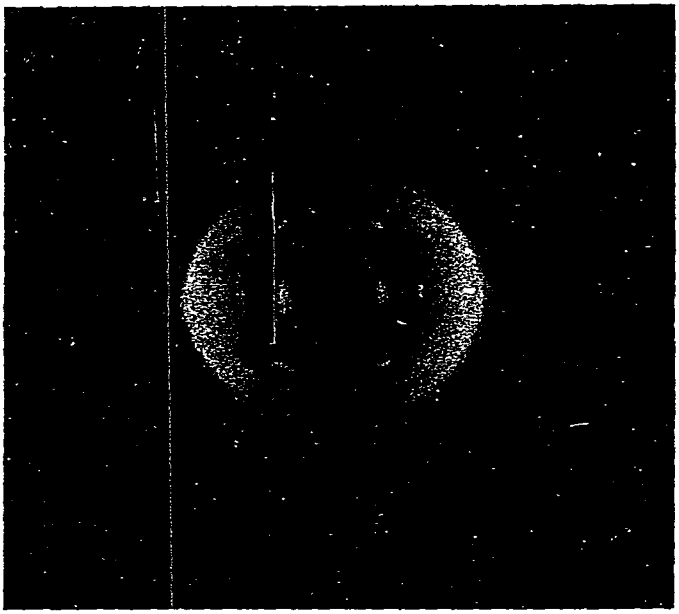

Fig. 1. Fibre diffraction diagram of the V_h-amylose–iodine complex.

ments, the diffracted X-ray intensities were collected on multiple film packs in an evacuated cylindrical camera of 5.73-cm radius. Ilford Industrial G film was used throughout. The intensities were measured, corrected, and converted into structure amplitudes as previously described[18]. CuKα radiation was used. The structure amplitudes for overlapping reflections were calculated as composites from $|F_c| = (\sum_i m_i F_{ci}^2)^{1/2}$, where m_i is the multiplicity, and the summation is carried out over all diffracting planes contributing to the composite. Unobserved intensities were assigned relative intensity values of one-half of the minimum observable intensity in that region of the scattering angle and weighted one-half in the calculation of a weighted *R* value.

The sixteen individual reflections observed on the fibre diagram were sufficient to identify the unit cell as orthorhombic with a = 13.60 Å, b = 23.42 Å, and c = 8.17 Å (fibre repeat). However, these data were insufficient to carry out a detailed refinement of the structure against X-ray data. Therefore, the Debye–Scherrer data collected previously by Zaslow and Miller[20] were used after being indexed with the orthorhombic unit-cell as determined from the fibre X-ray diagram. The Debye–Scherrer data were corrected for Lorentz and polarisation effects and then converted into structure amplitudes. Since only zero- and first-order layer lines were observed on the fibre diagram, only hkl indices with l = 0 or l = 1 were considered in indexing the Debye–Scherrer intensity envelopes. No separation was attempted between zero and first-order layer lines, *i.e.*, l = 0 or l = 1. Relative intensities measured by Zaslow and Miller agreed well with those present on the fibre diagram.

RESULTS AND DISCUSSION

The dimensions of the V-amylose unit-cell increase with increasing degree of hydration[6]. The anhydrous polymorph (V$_a$) exhibits an a axis of 13.0 Å; in the fully hydrated (V$_h$) form, the a axis is 13.7 Å. Amylose–iodine complexes can also occur in anhydrous and hydrated forms[20]. The structure investigated showed an a axis of 13.6 Å, indicative of a highly hydrated condition.

The space group of V$_a$- and V$_h$-amylose was previously determined as $P2_12_12_1$ with sections of two chains passing through the unit cell. Since the unit-cell volumes of V$_a$-amylose, V$_h$-amylose, and the amylose–iodine complex are similar (2304 Å^3 compared to 2604 and 2602 Å^3, respectively), we assumed that sections of two amylose chains also pass through the amylose–iodine unit-cell. Due to the possibility of having an odd number of iodide atoms in the fibre direction per fibre repeat, the space group $P2_1$ was chosen as most probable, with the only 2_1 screw-axis perpendicular to the a,c plane of the unit cell (second setting). This space group, $P2_1$, is included in $P2_12_12_1$, but of lower symmetry. Although the distribution of the iodine species (chain) might be at random in the centre of the amylose helix, this space group is nevertheless a good choice for the amylose helix itself and, as will be discussed later, also for the iodine species. Systematic absences of reflections agreed with space group

TABLE I

(a) CARTESIAN CO-ORDINATES (Å) OF THE FIRST RESIDUE OF V_h-AMYLOSE–IODINE IN THE REFERENCE POSITION[a]

Atom	X	Y	Z	*Atom*	X	Y	Z
O-4	0 000	−4 131	0 000	O-5	−3 570	−3 520	−0 472
C-1	−3 935	−3 341	0 878	O-6*gg*	−2 170	−4 827	−2 707
C-2	−3 240	−4 383	1 740	H-1	−4 974	−3 462	0 969
C-3	−1 731	−4 347	1 537	H-2	−3 591	−5 333	1 464
C-4	−1 388	−4 454	0 057	H-3	−1 350	−3 446	1 918
C-5	−2 158	−3 394	−0 725	H-4	−1 575	−5 419	−0 314
C-6	−1 964	−3 496	−2 227	H-5	−1 842	−2 445	−0 408
O-2	−3 586	−4 150	3 104	H-6A	−0 995	−3 179	−2 478
O-3	−1 128	−5 443	2 224	H-6B	−2 655	−2 861	−2 697

(b) CARTESIAN CO-ORDINATES (Å) OF IODINE AND WATER IN ONE ASYMMETRIC UNIT OF SPACE GROUP $P2_1$

I-1	3 696	−0 099	0 016	O-W1	3 125	−8 734	1 004
I-2	3 247	−0 231	2 889	O-W2	−4 548	−5 485	−0 180
I-3	3 311	0 202	5 768	O-W3	3 675	8 734	5 089
				O-W4	−2 262	5 485	3 905

(c) FRACTIONAL ATOMIC CO-ORDINATES FOR ONE RESIDUE OF THE 6_5 V_h-AMYLOSE–IODINE COMPLEX CHAIN AND THE WATER AND IODINE MOLECULES OF ONE ASYMMETRIC UNIT IN SPACE GROUP $P2_1$, THE VIRTUAL-BOND LENGTH (O-4 O-4_2) IS 4 35 Å

O-4	0 5381	0 0560	−0 3599	O-5	0 5787	−0 0969	−0 4176
C-1	0 5748	−0 1141	−0 2524	O-6*gg*	0 6372	−0 0225	−0 6912
C-2	0 6312	−0 0718	−0 1469	H-1	0 6075	−0 1545	−0 2412
C-3	0 5935	−0 0112	−0 1717	H-2	0 7057	−0 0732	−0 1807
C-4	0 5930	0 0041	−0 3529	H-3	0 5218	−0 0080	−0 1251
C-5	0 5370	−0 0414	−0 4486	H-4	0 6646	0 0096	−0 3983
C-6	0 5396	−0 0322	−0 6324	H-5	0 4635	−0 0415	−0 4098
O-2	0 6230	−0 0890	0 0201	H-6A	0 4949	0 0028	−0 6632
O-3	0 6559	0 0281	−0 0876	H-6B	0 5114	−0 0687	−0 6900
Iodine and water							
I-1	0 2718	−0 0042	0 0020	O-W1	0 2298	−0 3729	0 1229
I-2	0 2387	−0 0099	0 3536	O-W2	−0 3337	−0 2342	−0 0220
I-3	0 2435	0 0086	0 7060	O-W3	0 2702	0 3729	0 6229
				O-W4	−0 1663	0 2342	0 4780

---

[a] For proper positioning in the unit cell, the residue must be shifted 1/4 in *a* as is necessary for space group $P2_1$, in the second setting, rotated 108 5° around z, and shifted −2 94 Å along z The virtual bond length O-4 O-4_2 is 4 35 Å

$P2_1$. The amount of iodine taken up by amylose had previously been estimated as 26% by weight, corresponding to 2.2 iodide atoms per six glucosyl residues.

Conformation and packing analysis was performed by the method of stereochemical refinement analysis[19]. The left-handed, helical conformation of amylose that had been found in previous studies of V_a-amylose[22] and V-Me_2SO-amylose[21] was adjusted to the 8.17-Å helix pitch, as measured for V_h-amylose–iodine. It was found that the most convenient virtual-bond length O-4 $\cdots$ O-4_2 was 4.35 Å, compared to 4.28 Å in V_a-amylose[19]; for a discussion of virtual-bond terminology, see ref. 24. The glycosidic angle (C-1–O-4_2–C-4_2) was 120°, and two intramolecular hydrogen-bonds occur in this conformation: O-2 $\cdots$ O-3_2 of adjacent residues with length of 2.98 Å, and O-2 $\cdots$ O-6_7 of succeeding turns with 2.83 Å. The most stable rotamer of O-6 was in the vicinity of *gg** [τ(O-5–C-5–C-6–O-6) = 72°]. No non-bonded contacts occur below the cut-off distances[26]. The shortest H $\cdots$ H distances is H-1 $\cdots$ H-4_2 at 2.18 Å.

Helices having the conformation described above were packed stereochemically in the unit cell with helix rotations, helix translations, and all bond lengths, bond angles, and torsion angles as the variable parameters[19]. The starting model for

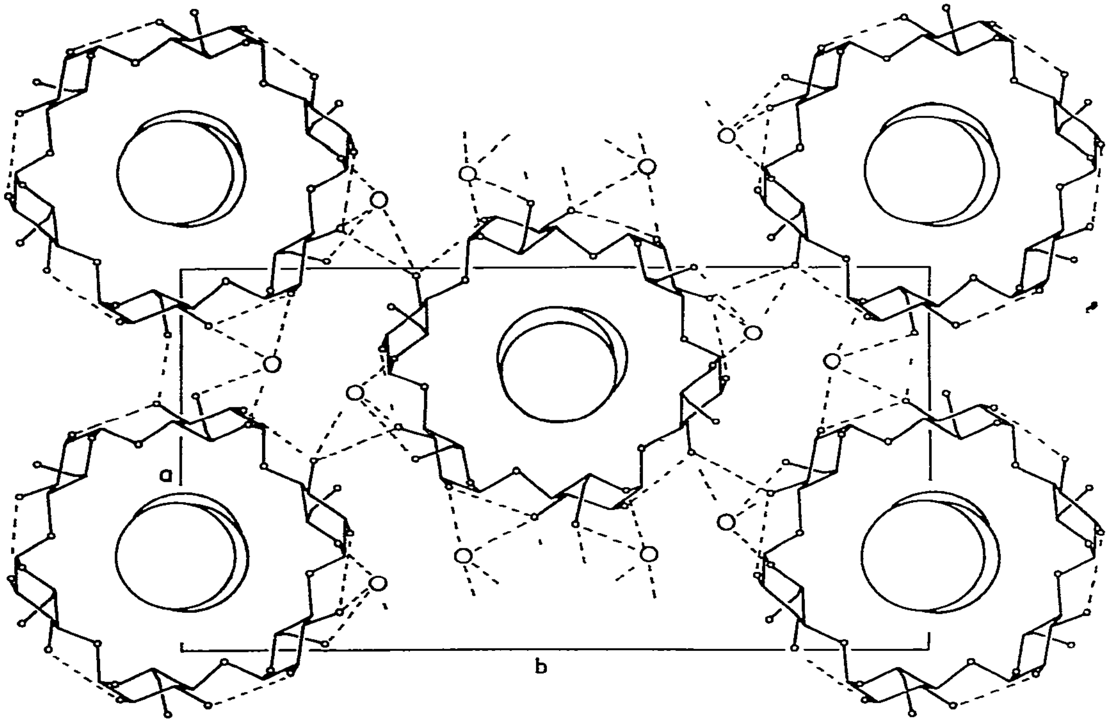

Fig. 2. View of the V_h-amylose–iodine complex in the *a,b* plane of the unit cell, showing the hydrogen-bonding arrangement.

---

*gg means *gauche* to both O-5 and C-4.

TABLE II

POSSIBLE INTRA- AND INTER-MOLECULAR HYDROGEN-BONDS[a] FOR THE V_h-AMYLOSE–IODINE COMPLEX

Hydrogen bond		*Distance (Å)*
Intra		
O-2	O-3_2	2 98
O-2	O-6-	2 83
Inter		
O-3_2	O-3_3^b	2 88
O-3	O-6^d	2 94
O-5	O-$W1^c$	2 74
O-6	O-$W1^c$	2 81
O-3	O-$W1^e$	2 81
O-2_3	O-$W1^b$	2 76
O-5_2	O-$W2^c$	2 63
O-6_3	O-$W2^c$	2 92
O-3_3	O-$W2^b$	2 73
O-3_2	O-$W2^f$	2 68

[a] As the packing refinement for the amylose helices with the associated water molecules has been performed in space group $P2_12_12_1$ the space group found for V_h-amylose only those hydrogen bonds are listed which are unique in this space group Note O-W3 and O-W4 are symmetry-related to O-W1 and O-W2 respectively [b] $1/2 + x$ $-1/2 - y$ $-z$ [c] $1/2 - x$ $-1/2 - y$ $-1 - z$ [d] $3/2 - x$ $-y$ $1/2 + z$, [e] $1 - x$ $1/2 + y$ $-1/2 - z$ [f] $1 - x$ y z

this refinement was that obtained for V_h-amylose[28] with 8 water molecules in the unit cell, which had been varied to form an optimal hydrogen-bonding scheme with the amylose helices The result of this analysis showed additional hydrogen bonds between the amylose helices between O-3 atoms of adjacent, antiparallel helices with a length of 2 88 Å, and O-3 O-6 of the parallel corner chains with 2 94 Å, and with water molecules as shown in Fig 2 and listed in Table II It should be noted that these packing calculations were carried out in the space group ($P2_12_12_1$) in which pure V_h-amylose crystallises This procedure was chosen to minimise the variables and to save computer time since the few reflections observed are not sufficient to test for space-group symmetry No real short-contacts were encountered

Next, the iodine species were located by trial and error Preliminary calculations were carried out in space group $P2_1$ second setting The iodide atoms were placed in all stereochemically possible positions in the unit cell with the best packing positions of the amylose helices The crystallographic R value, defined as $\sum w \big||F_o| - |F_c|\big| / \sum w |F_o|$ was calculated, where $|F_o|$ and $|F_c|$ are the observed and calculated structure amplitudes respectively, and w is a weight factor The data set of ref 20 and an isotropic temperature factor of $B = 5$ [temperature factor $= \exp(-B\sin^2\theta)$] were used Scattering factors for carbon, hydrogen, oxygen, and iodide were obtained from the International Tables for X-ray Crystallography[25] The R value calculated for the structure without iodide was ~0.55. Placement of the iodide atoms in the interstitial spaces served only to increase the *R* value. However, placement in the central channel of the helix resulted in decreased *R* values. A minimum in *R* was found with three iodide atoms in one turn of the amylose helix, *i.e.*, six iodide atoms per unit cell, which corresponds to an iodine uptake of 35% by weight, in reasonable agreement with previous estimates[5] of 26%.

This procedure for locating the iodine species can only be regarded as crude, since the fibre pattern of the amylose–iodine complex showed one, rather diffuse, meridional reflection at a *d*-spacing of 2.9 Å, which is incongruous with the layer-line spacing of 2.72 Å of the amylose helices. Therefore, if the iodine species do contribute to the scattering of the first layer line, a splitting of this layer line is to be expected due to the scattering of the amylose helices and the iodine species, respectively. However, such a splitting was not observed. This experimental evidence can be interpreted as indicating that polyiodide chains having an average spacing of 2.9 Å between the iodide atoms in the *z* direction are distributed at random inside the amylose helices. Such a distribution of polyiodide chains without the amylose helices will result in equatorial reflections and a meridional reflection of 2.9 Å only. Discrete spots will not be found on any higher layer lines. Unfortunately, no conclusions can be drawn about the lengths of the polyiodide chains.

Therefore, the strategy in placing the iodide atoms inside the helices was to use the equatorial reflections of the fibre pattern and the appearance of a single 2.9-Å meridional reflection as X-ray data for the refinement procedure. Two models have been tested: (*a*) the iodine chain has a three-fold symmetry or (*b*) a five-fold symmetry along the *z* axis, thus mimicking an I_3^- or I_5^- species or a longer polyiodide chain with this symmetry, respectively.

In a first step, the iodide atoms were placed in space group P1 in two independent channels with *c* dimensions corresponding to (*a*) 3 × 2.9 Å or (*b*) 5 × 2.9 Å, and their positions were calculated by *R*-factor refinement using the observed reflections. The amylose helices were not considered during this refinement. Only the missing and observed meridional reflections have been added, thus forcing the iodide atoms to the desired 2.9-Å repeat distance in *z*. An extreme anisotropic temperature-factor was used for this calculation. With this procedure, the iodide atoms can be located in three dimensions within the unit cell at places where the channels of the amylose helices were expected. The corresponding *R* values for model (*a*) and (*b*) were almost equal and no significant difference was detected upon adding the unobserved, equatorial structure amplitudes, indicating no preference for either of the two models. Therefore, model (*a*) was used in further calculations for reasons of simplicity. A symmetry relationship between the two independent iodine species was detected close to space group P2$_1$, second setting, with the 2$_1$ screw-axis perpendicular to the *a,c*, plane. Therefore, space group P2$_1$ was used in further calculations.

Next, the amylose helix with the water molecules was added, as obtained by conformation and packing analysis. A refinement of the iodide positions against the *R* factor and a combined refinement against *R* and packing was undertaken with the equatorial reflections in space group $P2_1$ No significant difference between the two refinement procedures was found, and a final value of 29% in the weighted R factor and 28% in $R2$ was obtained The weights used were one for observed reflections and one-half for unobserved reflections, $R2$ is the discrepancy factor for the squared difference in the structure amplitudes $R2 = \{\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2\}^{1/2}$ The shortest contacts between iodide and other atoms are hydrogen–iodide contacts of 3 3 Å A refinement of the co-ordinates of the amylose helix did not seem to be useful, in view of the few data and the uncertainty caused by the strong scattering of the iodine species Therefore, further tests were undertaken to check the results obtained Calculating the first layer line with 7 pairs of a total of 12 overlapping, observed and unobserved reflections for the V_h-amylose helices and the water positions only resulted in weighted values of $R = 36\%$ and $R2 = 43\%$ These high values are due to

TABLE III

OBSERVED AND CALCULATED STRUCTURE AMPLITUDES OF A FIBRE X-RAY DIAGRAM OF V_h-AMYLOSE–IODINE[a]

h k l	$\|F_c\|$	$\|F_o\|$
1 1 0 0 2 0	28	21
2 0 0 1 3 0	12	14
2 2 0, 0 4 0	28	43
3 1 0, 2 4 0, 1 5 0	78	95
3 3 0, 0 6 0	51	31
Unobserved F_0^2 taken as one-half of the observable intensity		
1 0 0	0	0
1 2 0	1	2
2 1 0	2	3
1 4 0	6	3
2 3 0	7	3
3 0 0	0	3
3 2 0	11	6
1 6 0	10	7
2 5 0	7	7
3 4 0	5	7
First layer line		
0 1 1	4	6
1 0 1, 1 1 1, 0 2 1	19	18
2 0 1 1 3 1, 2 1 1	9	18
2 2 1, 0 4 1	12	17
Unobserved		
1 2 1	14	2
0 3 1	4	3
1 4 1	7	4

[a] The zero layer line (equator) was calculated with iodide atoms, and the first layer line without iodide atoms Normalisation constants are 0 057 for the equator and 0 055 for the first layer line

a large, calculated (121) reflection The *R* values for the observed reflections only were $<30\%$ When the iodide atoms were added, the *R* values increased considerably Use of the 147 pieces of data from the Debye–Scherrer experiment[20], where no separation of the zero and first layer line can be obtained, resulted in $R = 31\%$ and $R2 = 31\%$ The calculated and observed structure amplitudes for the fibre pattern are listed in Table III

Cartesian co-ordinates of the best-refined glucosyl residue by packing and conformation analysis, the water molecules, and the iodide atoms are given in Table I, and possible hydrogen bonds in Table II Note that the iodide-chain repeat distance in z is different from that for the amylose helix The projection of the unit cell onto the *a,b* plane is shown in Fig 2

CONCLUSION

A comparison of the V_a-amylose structure and the V_h-amylose–iodine structure is of interest In V_a-amylose, the fibre repeat is 0 26 Å shorter than the 8 17-Å repeat of V_h-amylose–iodine HO-6 Groups were found at three different rotameric positions, *gt*, *gg*, and *tg*, in V_a-amylose[19], resulting in a true 2_1 screw-axis along the polymer chain However, the HO-6 rotameric positions in V_h-amylose–iodine were all *gg* and along with the three iodide atoms in one turn of the helix, resulted in the absence of a 2_1 screw-axis in the helix direction The helix rotational position in V_a-amylose is 88 6° from the reference position (O-4 at 0, $-y_0$,0), in V_h-amylose–iodine, it is 108 5° The helix translation in V_a-amylose is —3 98 Å and in V_h-amylose–iodine it is —4 98 Å, with both helices in equivalent positions and the same space group Therefore, the transition from V_a-amylose to V_h-amylose–iodine occurs with a helix rotation of 20° and a translation of 1 Å Water molecules are found between the helices in V_h-amylose–iodine and account for the expansion of the unit cell upon hydration The water molecules are hydrogen-bonded to the hydroxyl groups on the amylose helices in a dense network forming bridges between the chains

Iodide is found as a polyiodide chain in the helix channels with nearly equal separation distances between the atoms The distances are 2 91, 2 91, and 2 99 Å The length of the iodide chain cannot be determined The I I I angles are all nearly linear

ACKNOWLEDGMENTS

This work was supported by grants from Deutsche Forschungsgemeinschaft and from NATO The X-ray fibre diffraction patterns were obtained at the State University of New York College of Environmental Science and Forestry in the laboratory of Dr Anatole Sarko The computations were performed at the Computing Centre of the University of Freiburg

REFERENCES

1 R E Rundle and F C Edwards, *J Am Chem Soc*, 65 (1943) 2200–2203
2 R R Baldwin R S Bear and R E Rundle, *J Am Chem Soc*, 66 (1944) 111–115
3 R E Rundle and R R Baldwin *J Am Chem Soc*, 65 (1943) 554–558
4 R E Rundle J F Foster and R R Baldwin, *J Am Chem Soc*, 66 (1944) 2116–2120
5 R E Rundle and D French, *J Am Chem Soc*, 65 (1943) 1707–1710
6 R E Rundle *J Am Chem Soc*, 69 (1947) 1769–1772
7 R S Stein and R E Rundle, *J Chem Phys*, 16 (1948) 195–207
8 R Bersohn and I Isenberg *J Chem Phys* 35 (1961) 1640–1643
9 C D West, *J Chem Phys*, 15 (1947) 689
10 C D West, *J Chem Phys*, 19 (1951) 1432
11 R E Rundle *J Chem Phys*, 15 (1947) 880
12 C S Hanes, *New Phytol*, 36 (1937) 189
13 K Freudenberg, E Schaef, G Dumpert, and T Ploetz, *Naturwissenschaften*, 27 (1939) 850–853
14 F Cramer *Naturwissenschaften* 38 (1951) 188
15 J M Reddy, F Knox, and M B Robin, *J Chem Phys*, 40 (1964) 1082–1089
16 M Noltemeyer and W Saenger *Nature (London)* 259 (1976) 629–632
17 T L Bluhm and P Zugenmaier *Prog Colloid Polym Sci*, 64 (1978) 132–138
18 T L Bluhm and P Zugenmaier, *Polymer* 20 (1979) 23–30
19 P Zugenmaier and A Sarko *Biopolymers* 15 (1976) 2121–2139
20 B Zaslow and R L Miller *J Am Chem Soc* 83 (1961) 4378–4381
21 W T Winter and A Sarko, *Biopolymers*, 13 (1974) 1461–1482
22 W T Winter and A Sarko, *Biopolymers* 13 (1974) 1447–1460
23 T L Bluhm G Rappenecker, and P Zugenmaier *Carbohydr Res* 60 (1978) 241–250
24 A Sarko and R H Marchessault, *J Am Chem Soc*, 89 (1967) 6454–6462
25 International Tables for X-ray Crystallography Vol III Kynoch Press Birmingham 1962 p 202
26 G N Ramachandran C Ramakrishnan, and V Sasisekharan in G N Ramachandran (Ed), *Aspects of Protein Structure* Academic Press, London and New York, 1963, pp 121–135
27 R C Teitelbaum S L Ruby and T J Marks, *J Am Chem Soc*, 100 (1978) 3215–3217
28 G Rappenecker and P Zugenmaier, *Carbohydr Res*, 89 (1980) 11–19