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DETAILED REFINEMENT OF THE CRYSTAL STRUCTURE OF V_h-AMYLOSE*†

GERHARD RAPPENECKER AND PETER ZUGENMAIER**

Institut für Makromolekulare Chemie der Universität Freiburg D-7800 Freiburg i Br (German Federal Republic)

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ABSTRACT

The crystal structure of V_h-amylose has been refined by a combined analysis of stereochemical conformation and packing, and by analysis of X-ray fibre diagrams The unit cell is orthorhombic with a = 13 65 Å, b = 23 70 Å, and c (fibre repeat) = 8 05 Å The chain conformation is a left-handed six-fold helix with O-6 in the *gg* (*gauche* to O-5 and *gauche* to C-4) position The water molecules are located inside the helix channel of the amylose and in the interstitial spaces of the helices forming an intensive hydrogen-bonding network

INTRODUCTION

Amylose crystallises in polymorphic forms Native amylose occurs in A and B forms, both forming double helices[1] Single helices are obtained when amylose crystals are prepared from complexes with dimethyl sulfoxide[2] or with 1-butanol[3] These structures occur in a dry and hydrated state, and are denoted V_d- and V_h-amylose, respectively Intermediate forms are also known

In 1974, Zaslow *et al*.[4] reported on the structure of the V-amylose–H_2O system Besides an unacceptably high reliability index, the water molecules were not located and it was concluded that oxygen–oxygen (O-2 O-2) contacts of two adjacent chains are in part responsible for the larger packing diameter of V_h-amylose as compared to V_d-amylose In continuing our studies of the structure of polysaccharide–solvent complexes[5] and in order to obtain a starting model for the similarly packed V_h-amylose–iodine complex, where only a few reflections are present, we undertook a structural refinement of V_h-amylose and located the water molecules in this structure Such a determination was possible by applying the constrained optimisation procedure with simultaneous use of stereochemical criteria and X-ray data[6]

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*Conformation and Packing Analysis of Polysaccharides and Derivatives, Part VIII For Part VII, see preceding paper

†Dedicated to Professor G Rehage on the occasion of his 60th birthday

**To whom correspondence should be addressed Present address Institut fur Physikalische Chemie der T U Clausthal, 3392 Clausthal-Zellerfeld, German Federal Republic

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EXPERIMENTAL

V_h-Amylose fibres were prepared by casting amylose films from a 15% (w/w) solution in Me_2SO Strips of these films were then stretched in high humidity at room temperature (to an extension of 200 to 600%) and gave the oriented X-ray diagram of the Me_2SO–amylose complex[2] Treatment of these fibres, still held under tension, with boiling methanol for 20 min gave V_a-amylose fibres These fibres were kept under tension and placed in a chamber at 100% relative humidity, and converted into V_h-amylose An X-ray diagram of such a V_h-amylose fibre is shown in Fig 1

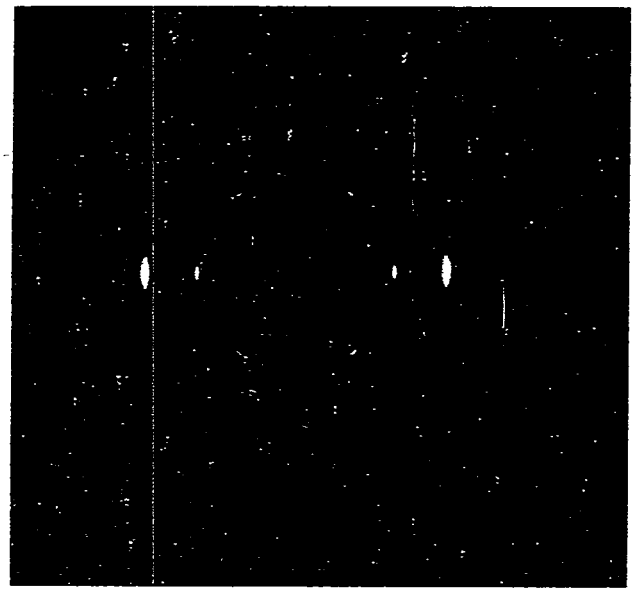

Fig 1 Fibre diffraction diagram of V_h-amylose taken with CuKα radiation in a cylindrical camera

X-Ray diffractograms were recorded on flat films for *d*-spacing measurements and on multiple film packs in a cylindrical camera for intensity measurements The data were processed as described previously[5 7] All reflections on the V_h-amylose fibre diagram can be indexed with an orthorhombic unit-cell with $a = 13\,65$ Å, $b = 23\,70$ Å and $c = 8\,05$ Å (fibre repeat), and were obtained by least-squares refinement These values agree well with those given by Zaslow *et al*[4] Space group $P2_12_12_1$ is suggested by the extinct reflections

STRUCTURE DETERMINATION

The simultaneously performed stereochemical conformation and packing analysis has been described[6 7] Here, three different kinds of disagreement indices

have been introduced into the X-ray refinement a weighted R_w factor, $R_w = \sum w \left||F_o| - |F_c|\right| / \sum w |F_o|$, with the weight w set equal to 1 for observed reflections and 0.5 for unobserved reflections, $|F_o|$ and $|F_c|$ are the observed and calculated structure amplitudes, the structure amplitudes of unobserved reflections were assigned one-half of the minimum observable intensity in the corresponding region of diffraction

TABLE I

(*a*) CARTESIAN CO-ORDINATES (Å) FOR ONE RESIDUE OF THE 6_5 V_h-AMYLOSE HELIX IN THE STANDARD POSITION[a] [O-4 AT (0, −10, 0), VIRTUAL-BOND LENGTH 4.30 Å]

Atom	X	Y	Z	*Atom*	X	Y	Z
O-4	0.000	−4.085	0.000	H-1	−4.962	−3.468	1.133
C-1	−3.928	−3.359	0.988	H-2	−3.519	−5.311	1.670
C-2	−3.185	−4.339	1.885	H-3	−1.338	−3.332	1.942
C-3	−1.687	−4.276	1.643	H-4	−1.522	−5.463	−0.134
C-4	−1.368	−4.467	0.162	H-5	−1.915	−2.522	−0.454
C-5	−2.213	−3.504	−0.673	H-6A	−1.107	−3.448	−2.470
C-6	−2.071	−3.729	−2.163	H-6B	−2.770	−3.124	−2.661
O-2	−3.496	−4.041	3.237	O-4(2)	−3.538	−2.042	1.342
O-3	−1.025	−5.276	2.421				
O-5	−3.610	−3.639	−0.360				
O-6	−2.308	−5.086	−2.524				

[a]The residue of the amylose helix has to be rotated 109.6° around z and shifted −5.25 Å along z to be in the best position. The asymmetric unit consisting of three residues has to be shifted by 1/4 in a for space group $P2_12_12_1$

(*b*) CARTESIAN CO-ORDINATES (Å) OF THE WATER MOLECULES OF ONE ASYMMETRIC UNIT

O-W1	3.297	−8.843	−0.818	O-W3	2.965	−0.668	−3.672
O-W2	−4.511	−5.482	−2.017	O-W4	2.796	−1.285	−6.297

(*c*) FRACTIONAL ATOMIC CO-ORDINATES FOR ONE RESIDUE OF THE 6_5 V_h-AMYLOSE HELIX (VIRTUAL-BOND LENGTH 4.30 Å) AND THE WATER MOLECULES OF ONE ASYMMETRIC UNIT

Atom	x	y	z	*Atom*	x	y	z
O-4	0.5319	0.0578	−0.6522	H-1	0.6113	−0.1481	−0.5114
C-1	0.5784	−0.1086	−0.5294	H-2	0.7030	−0.0647	−0.4447
C-2	0.6277	−0.0652	−0.4180	H-3	0.5128	−0.0060	−0.4109
C-3	0.5866	−0.0065	−0.4481	H-4	0.6644	0.0168	−0.6688
C-4	0.5919	0.0088	−0.6320	H-5	0.4711	−0.0404	−0.7086
C-5	0.5462	−0.0384	−0.7358	H-6A	0.5152	0.0048	−0.9590
C-6	0.5583	−0.0295	−0.9209	H-6B	0.5337	−0.0659	−0.9827
O-2	0.6148	−0.0818	−0.2501	O-4(2)	0.4779	−0.1117	−0.4855
O-3	0.6393	0.0339	−0.3514				
O-5	0.5899	−0.0920	−0.6969				
O-6	0.6577	−0.0198	−0.9657				
Water molecules							
O-W1	0.2415	−0.3731	−0.1016	O-W3	0.2172	−0.0282	−0.4561
O-W2	−0.3305	−0.2313	−0.2506	O-W4	0.2048	−0.0542	−0.7822

angle When all weights are set to one, the disagreement index R is obtained and the index $R2$ is defined by the weighted, squared difference of the structure amplitudes, $R2 = \{\sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2\}^{1/2}$

The structure of V_a-amylose[6] served as a preliminary model with an assumed 6_5 helix First, a stereochemical analysis of the conformation with the longer helix pitch for V_h-amylose was performed, followed by a preliminary refinement of R

TABLE II

POSSIBLE HYDROGEN BONDS AND SHORTEST VAN DER WAALS CONTACTS

Atoms			*Distance (Å)*
Hydrogen bonds			
Intramolecular			
O-2	O-3(2)	A[a]	2 84
O-2	O-6(7)	A	2 78
Intermolecular			
O-5	O-W1	C	2 76
O-6	O-W1	C	2 84
O-3	O-W1	I	2 76
O-2(3)	O-W1	D	2 80
O-5(2)	O-W2	C	2 66
O-6(3)	O-W2	C	2 90
O-3(3)	O-W2	E	2 70
O-3(2)	O-W2	B	2 75
O-W3	O-W4	A	2 70
O-W3	O-W4	H	2 63
O-3	O-6	G	2 94
van der Waals contacts			
Intramolecular			
O-2	C-6(7)	A	3 03
O-5	C-4(2)	A	3 33
O-2	H-6B(7)	A	2 45
H-5	O-4(2)	A	2 47
H-1	H-4(2)	A	2 12
Intermolecular			
C-2(2)	O-6(3)	C	3 27
C-6(3)	O-W4	A	3 30
O-6(2)	O-3(3)	C	3 40
H-2(2)	O-6(3)	C	2 31
O-6(2)	H-2(3)	C	2 32
H-5(3)	O-W3	A	2 48
H-6A(3)	O-W4	A	2 53
H-1(3)	O-W1	D	2 71
O-6	H-2	F	2 76
H-4(2)	H-4(3)	C	2 70

[a]Key A, x, y, z, B, $1 + x, y, z$, C, $1/2 + x, -1/2 - y, -1 - z$, D, $-1/2 + x, -1/2 - y, -z$, E, $1/2 + x, -1/2 - y, -z$, F, $3/2 - x, -y, -1/2 + z$, G, $3/2 - x, -y, 1/2 + z$, H, $1/2 - x, -y, 1/2 + z$, I, $1 - x, 1/2 + y, -1/2 - z$

and *R2* with helix rotation and translation using only the observed reflections in space group $P2_12_12_1$ This analysis led to the starting model for refinement calculations Two quite different helix positions were obtained when the refinement procedure was performed against *R* or *R2*, respectively This difference disappeared when the water molecules were introduced, which was done in the next step The search for possible water positions was started in the *a,b* projection followed by a three-dimensional analysis, as previously described[6], using X-ray data only Four water molecules per asymmetric unit were located, two inside the amylose helix channel and two in interstitial spaces of the amylose helices This search was performed with

TABLE III

BOND LENGTHS, BOND ANGLES AND TORSION ANGLES FOR V_h-AMYLOSE

Bond lengths	*(Å)*	*Δ*[a]	*Bond angles*	*(degrees)*	*Δ*
O-4–C-4	1 430	0 004	O-4–C-4–C-3	106 0	0 5
C-4–C-3	1 527	0 004	O-4–C-4–C-5	107 4	−1 2
C-4–C-5	1 529	0 004	C-3–C-4–C-5	109 6	−0 7
C-1–C-2	1 522	−0 001	C-4–C-5–O-5	111 0	1 0
C-3–C-2	1 519	−0 002	C-4–C-3–C-2	110 8	0 3
C-1–O-5	1 413	−0 001	C-3–C-2–C-1	111 1	0 6
C-5–O-5	1 438	0 002	C-5–O-5–C-1	114 1	0 1
C-1–O-4(2)	1 418	0 003	O-5–C-1–O-4(2)	111 1	−0 5
C-2–O-2	1 419	−0 004	C-2–C-1–O-4(2)	108 4	0 0
C-3–O-3	1 430	0 001	C-2–C-1–O-5	109 0	−0 2
C-5–C-6	1 514	−0 000	C-3–C-2–O-2	111 1	0 3
C-6–O-6	1 424	−0 003	C-1–C-2–O-2	108 6	−0 7
			C-4–C-3–O-3	110 1	0 4
			C-2–C-3–O-3	109 9	0 3
			C-4–C-5–C-6	113 1	0 4
			O-5–C-5–C-6	106 9	0 0
			C-5–C-6–O-6	112 1	0 3
			C-1–O-4(2)–C-4(2)	118 6	

Torsion angles	*(degrees)*	*Δ*
O-5–C-1–C-2–C-3	56 8	0 8
C-1–C-2–C-3–C-4	−53 6	−0 4
C-2–C-3–C-4–C-5	51 3	−1 7
C-3–C-4–C-5–O-5	−53 5	1 9
C-4–C-5–O-5–C-1	60 6	−0 5
C-5–O-5–C-1–C-2	−61 1	1 1
O-5–C-5–C-6–O-6	−69 8	
O-4–C-4–C-5–O-5	−168 3	
C-5–O-5–C-1–O-4(2)	58 4	
H-1–C-1–O-4(2)–C-4(2)	−14 4	
C-1–O-4(2)–C-4(2)–H-4(2)	−7 5	

[a] *Δ* Difference from the mean values given in ref 8

the rotational positions of O-6 either all *gt** or all *gg*. a preference was found for an all-*gg* conformation Next, an *R*-factor refinement was performed for the entire amylose helix and the water molecules with the obtained co-ordinates Hydrogen bonds were indicated by O O distances of ~3 Å or less Possible hydrogen-bonds were tested by a combined packing and *R*-factor refinement[6] The last refinement calculation was performed with all bond lengths, bond angles, torsion angles, and helix rotation and translation as variables This refinement procedure resulted in a helix conformation of which the co-ordinates for one D-glucosyl residue are listed in Table I It should be noted that an optimal hydrogen-bonding network for the

TABLE IV

CALCULATED AND OBSERVED STRUCTURE AMPLITUDES FOR V_h-AMYLOSE

h	k	l	$\|F_c\|$	$\|F_o\|$	h	k	l	$\|F_c\|$	$\|F_o\|$
0	1	0			1	2	1	256	111
1	2	0	119	111	2	0	1		
2	0	0			2	1	1		
1	3	0	436	468	1	3	1	164	184
2	2	0			0	4	1		
0	4	0	99	95	2	2	1	267	262
1	5	0			2	3	1		
2	4	0			1	4	1	221	223
3	2	0			3	1	1		
3	1	0	581	769	3	2	1		
0	6	0			2	4	1		
2	5	0			1	5	1	232	229
3	3	0	407	529	3	3	1		
2	6	0			2	5	1		
4	0	0			0	6	1		
4	1	0	136	123	1	6	1	214	284
1	7	0			1	0	2		
4	2	0			0	1	2		
3	5	0	175	279	1	1	2		
0	1	1	111	139	0	2	2	162	217
1	1	1			2	0	2		
0	2	1	280	279	2	1	2		
					0	3	2		
					1	3	2	203	223
Unobserved reflections, F_o^2, taken as one-half of the observable intensity									
1	2	0	29	33	1	0	1	137	50
2	1	0	30	45	0	3	1	76	45
1	4	0	75	56	2	1	1	36	45
2	3	0	172	56	0	5	1	114	56
1	6	0	146	73	3	0	1	102	61
3	4	0	139	78	1	2	2	45	56

*gt means *gauche* to O-5 and *trans* to C-4 and similarly for *gg* and *tg*

water molecules was assumed All possible hydrogen bonds and the shortest van der Waals contacts are given in Table II The short O-6 H-2 contacts can be easily lengthened to 2 46 Å when a pure conformation and packing analysis was performed An increase in R by 1% was encountered In Table III, the calculated bond lengths, angles, and torsion angles for V_h-amylose are listed and compared with the mean values given by Arnott and Scott[8]

RESULTS AND DISCUSSION

The following R values were obtained for the final model with the combined X-ray and stereochemical refinement-procedure R_w = 21%, R = 26%, and $R2$ = 25%, with a total of 46, mostly overlapped, observed reflections and 12 unobserved reflections The observed and calculated structure amplitudes are listed in Table IV An isotropic temperature factor of B = 5 was used throughout the refinement calculations The hydrogen-bonding network and the amylose helices are shown in the a,b plane projection of the unit cell in Fig 2

The use of different R values in the refinement procedure resulted in structures that were insignificantly different The lowest R values were obtained with all HO-6

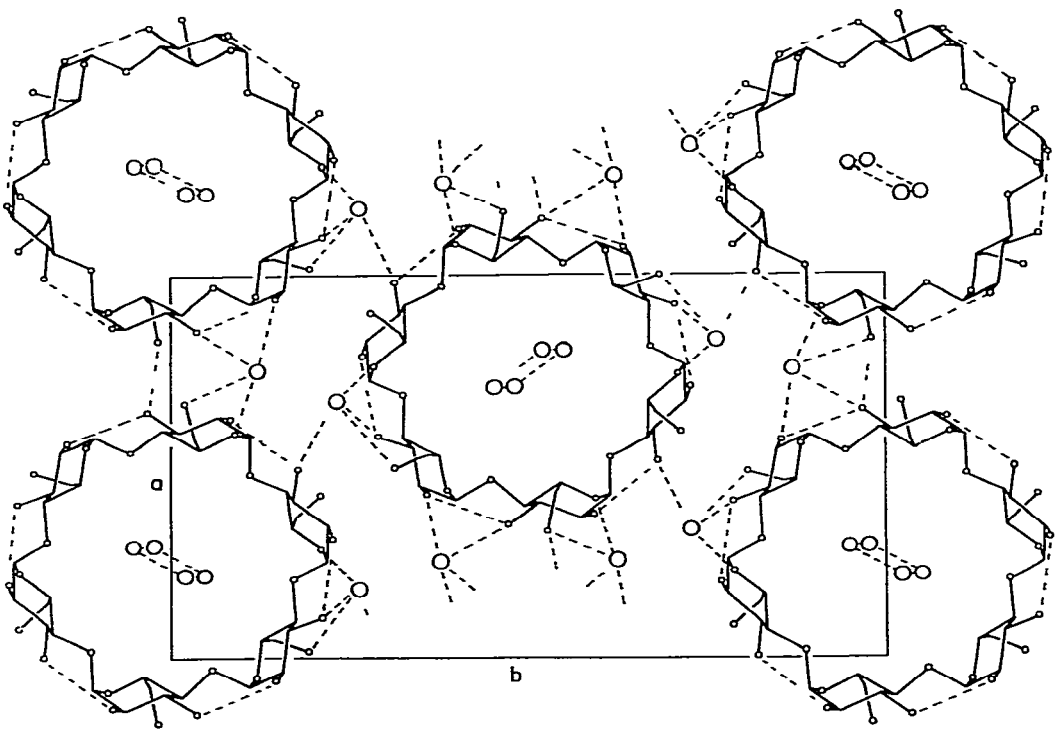

Fig 2 View of V_h-amylose in the a,b plane of the unit cell, showing the hydrogen-bonding arrangement

groups in the vicinity of the *gg* conformation An all-*gt* rotational position of O-6 increased all three R values by 12% Even when only one O-6 of the asymmetric unit of space group $P2_12_12_1$ was in the *gt* position the R values changed to significantly higher values With O-6 in an all-*tg* rotational conformation, there was an increase of 18% in all three R values, and one O-6 in the *tg* position also increased the R values

The glycosidic angle at O-4 is 118 6° The torsion angles at the glycosidic linkage ϕ[H-1 C-1 O-4(2)* C-4(2)] and ψ[C-1 O-4(2) C-4(2) H-4(2)] are −14 4° and −7 5°, respectively as compared to 15 5° and −5 1° in ref 4 The optimal, virtual bond-length [O-4 O-4(2)] is 4 30 Å

CONCLUSIONS

Using knowledge of the crystal structure of V_a-amylose, we are now able to report the transition occurring with the transformation V_a-amylose ↔ V_h-amylose The space group remains $P2_12_12_1$, but the number of water molecules increases from 4 to 16 per unit cell The unit-cell volume increases from 2304 to 2604 Å^3 However, "hexagonal" packing is found in both structures In V_a-amylose, HO-6 adopts three different rotational positions close to *gt*, *gg*, and *tg* on successive residues, whereas, in V_h-amylose, O-6 adopts only one rotational position close to *gg* Water molecules are found in V_h-amylose inside the helix channel and in the interstitial spaces of adjacent chains, and give rise to an increase in the unit-cell dimensions in a and b The difference in the c dimension of 0 14 Å on going from V_a- to V_h-amylose may be due to a different intrachain hydrogen-bonding or to the placement of water inside the helix channel The shortest oxygen–oxygen contact [O-6(2) O-3(3) = 3 4 Å] of adjacent chains occurs between corner and antiparallel centre chains, which cannot be regarded as responsible for an increase in the unit-cell dimensions The proposed O-2 O-2 contact[+] is even longer and insignificant

A difference in helix rotation and helix shift in c of 21° and 1 3 Å has been detected between the two polymorphs and is significantly different from that noted previously[+] The water molecules in the interstitial spaces have been forced into positions with an optimal number of hydrogen bonds in a dense network as found in cyclohexa-amylose complexes[9] However, a slight change in the water positions, still inside the detectable range of the X-ray refinement technique, will result in fewer hydrogen bonds The two water molecules inside the helix channel are linked by hydrogen bonds, but are not hydrogen-bonded to any oxygen atom of the amylose helix, which might be possible with O-6 in the *gt* position However, the rotational position of all HO-6 groups is close to *gg*, in contrast to the proposal of Zaslow *et al*[+] where an all-*gt* position was assumed, or to the results obtained by a single crystal determination of cyclohexa-amylose where a mixture of O-6 in *gg* and *gt* rotational positions was observed[9] Here, the water molecules inside the macro-ring were

*In the numbering of atoms in this paper the number of the residue is indicated in parenthesis

hydrogen-bonded to some of O-6 *gt* The question of whether this difference in the structures of cyclohexa-amylose and V_h-amylose is due to the continuous nature of the amylose helix cannot be answered at the present

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