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CRYSTAL STRUCTURE OF THE KOH–AMYLOSE COMPLEX*

ANATOLE SARKO† AND ALAN BILOSKI

Department of Chemistry, SUNY College of Environmental Science & Forestry, Syracuse Campus, Syracuse, New York 13210 (U.S.A.)

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ABSTRACT

The crystal structure of potassium hydroxide complexed amylose, obtained by heterogeneous deacetylation of amylose triacetate, has been determined through a combined stereochemical structure-refinement and X-ray diffraction-analysis. The structure crystallizes in an orthorhombic unit-cell with parameters $a = 8.84, b = 12.31$, and c (fiber repeat) $= 22.41$ Å, and with $P2_12_12_1$ symmetry. The conformation of the amylose chain is a distorted, left-handed helix with 6 D-glucose residues per turn. Each three-residue asymmetric unit is complexed with one molecule of potassium hydroxide and three molecules of water. The K^+ ion coordinates with four oxygen atoms of the amylose chain and with two other oxygen atoms, and this coordination is probably the cause for the more-extended amylose chain-conformation than would be predicted from a ϕ,ψ map. The distortions in the chain are primarily manifested by different O-6 rotations and by slightly different bridge and ϕ,ψ angles for the individual residues. The structure is extensively hydrogen bonded, although largely through water molecules, which accounts for its ready water solubility. The left-handed conformation of the chain in this structure is consistent with the conformations of amylose triacetate and V-amylose, both of which are left-handed.

INTRODUCTION

When a fiber of amylose triacetate is deacetylated under tension with weak, alcoholic alkali, a crystalline, well-oriented fiber of alkali–amylose results. Its X-ray diffraction-diagram contains a relatively large number of reflections, and its fiber repeat is consistent with a helical structure of 6 D-glucose residues per turn. Senti and Witnauer were the first to show that its unit cell is orthorhombic and contains two helices, and that the chemical analysis of the fiber is consistent with 4 molecules of alkali and 12 molecules of water per cell[1,2]. The same authors also showed that the diffraction patterns of alkali–amyloses generated by the use of such different alkalis as LiOH, KOH, NaOH, and CsOH, are isomorphous[2].

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†To whom correspondence should be addressed.

Alkali–amylose is one of several polymorphic forms in which amylose crystallizes. Washing with alcohol converts it into V-amylose, a structure that we have previously shown to be a left-handed, 6-fold helix[3,4]. If the alkali is carefully removed with aqueous alcohol and the fiber is then subjected to a humidification regime, either A- or B-amylose can be obtained, according to the method of humidification[1,5,6]. The structure of A- and B-amyloses have been shown to be double-stranded helices[5,6]. Other polymorphs are similarly prepared, for example, alkali amylose may readily be converted into different salt complexes[7].

Because of the extensive polymorphism of amylose, and the central position occupied in it by alkali–amylose, the determination of its detailed crystal structure became of interest to us. The handedness of the helix was of primary interest, as it is known that amylose triacetate from which alkali–amylose is obtained is left-handed[8], whereas A- and B-amyloses into which it can be converted are right-handed[5,6]. Other points of interest concerned the effect of the alkali on the chain conformation and the possible presence of distortions in the helix. The latter are suggested by the presence of a second-order meridional in the diffraction diagram that is not consistent with 6-fold molecular symmetry.

EXPERIMENTAL

Amylose triacetate was prepared from potato amylose of $\bar{M}_w$ = 750,000 daltons (courtesy of Dr. F. R. Dintzis, USDA Laboratories, Peoria, IL) by the method of Jeanes and Jones[9]. It was cast into film and stretched into a crystalline fiber as previously described[8]. The fiber was deacetylated with 2% potassium hydroxide (75% methanol) at ~25° as described by Senti and Witnauer[1,2]. X-Ray diffraction patterns were recorded on Ilford Type G X-ray film in an evacuated, pin-hole camera by using CuKα radiation. The density of the fiber was determined by flotation in a mixture of carbon tetrachloride and dichloromethane.

The interplanar spacings were measured on diagrams obtained with sodium fluoride-calibrated samples, and the unit-cell parameters were refined against selected *d*-spacings by least-squares methods. Intensities of all discernible reflections were measured, corrected, and converted to $|F|_{obs}$ as previously described[10].

All calculations were performed with a CDC-3200 computer.

RESULTS

Diffraction measurements. — The X-ray diffraction diagrams of potassium hydroxide–amylose obtained by us appeared identical with the diagram previously published by Senti and Witnauer[1]. A total of 56 individual reflections, lying on eight layer-lines could be measured and indexed, resulting in an orthorhombic unit-cell having dimensions a = 8.84, b = 12.31, and c (fiber repeat) = 22.41 Å. These dimensions differ very slightly from those previously reported by Senti and Witnauer[1].

The calculated and observed *d*-spacings are listed in Table I*. The systematic absences of reflections in the X-ray pattern suggest a $P2_12_12_1$ space group, in agreement with the findings of Senti and Witnauer[2]. The intensities of 57 diffraction maxima located on the equator and five layer-lines could be reliably resolved and measured. These intensities contained contributions from 79 *hkl* planes. In addition, 20 unobserved reflections were assigned half of the minimum observable intensity in the corresponding diffraction-angle region.

The experimental density (1.55 g/cc) is consistent with 12 glucose residues, 4 potassium hydroxide units and 8–12 molecules of water per unit cell, which yields a theoretical density-range of 1.57–1.62 g/cc. A cell containing 12 glucose residues, 8 potassium hydroxide units, but no water has a calculated density of 1.63 g/cc and is thus also possible. As already discussed by Senti and Witnauer, the observed unit-cell dimensions and space-group symmetry are consistent only with two helices per cell, one at the corner and the other at the center of the cell, each helix containing six residues per turn[2]. Patterson maps calculated with observed intensities are in agreement with this arrangement.

Chain conformation and packing. — Initial chain-conformations for both left- and right-handed 6-fold helices were constructed by utilizing the Arnott–Scott average glucose residue[11]. These conformations were then refined against stereochemical criteria (namely, most probable bond-lengths, bond-angles, and torsion-angles) by refinement procedures previously described[4,12]. The sixfold symmetry of the helix was maintained in these refinements and the geometry of the glucose residue was varied through a wide range of the distance between successive glycosidic oxygen atoms or the *virtual bond* (*VB*). During these conformational refinements, it became apparent that helices having *VB* in the range 4.30–4.60 Å constituted the most-probable conformations, and that both left- and right-handed helices were approximately equally probable.

Intramolecular hydrogen-bond formation was not allowed by helices of either handedness. All three of the most-probable rotational positions for the 6-hydroxymethyl group, namely, O-6 in *gg*, *tg*, and *gt* positions, were possible[15].

The packing of the most-probable helix conformations was then determined by simultaneously refining the chain conformation and the positions of the chains in the unit cell against the stereochemical criteria previously used, but now including interchain, nonbonded contacts. The details of the refinement method have been previously described[4,12]. Initially, 6-fold helix symmetry was retained and the $P2_12_12_1$ space-group symmetry was maintained. All attempts to refine chain positions independently by eliminating symmetry between them always resulted in both chains moving back to $P2_12_12_1$ symmetry. Accordingly, symmetry was maintained in all subsequent refinements and parallel chain-packing was not considered any further.

*Tables I and III are deposited with, and can be obtained from, Elsevier Scientific Publishing Company, BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/123/*Carbohydr. Res.*, 79 (1980) 11–21.

TABLE II

CHARACTERISTICS OF THE BEST PACKING AND BEST X-RAY STRUCTURES[a]

Model	VB *length (Å)*	O-6 *Rotational positions (deg.)*	*Glycosidic bridge angles (deg.)*	*Rotational and translational position of helix (deg., Å)*	*Conformational angles ϕ,ψ (deg.)*	R″
Left-handed, six-fold molecular symmetry (except O-6, best packing agreement)	4.500	O-6_1: −60 (*gg*) O-6_2: −50 (*gg*) O-6_3: 180 (*tg*)	114.3	31.9, 7.48	−27.1, −32.4	—
As above, but best X-ray agreement (Includes KOH and H_2O)	4.500	O-6_1: −55 (*gg*) O-6_2: −55 (*gg*) O-6_3: −175 (*tg*)	114.5	33.9, 7.52	−26.2, −32.9	0.328
Left-handed, independent residues, best X-ray agreement (Includes KOH and H_2O)	Res. 1: 4.500 Res. 2: 4.501 Res. 3: 4.528	O-6_1: −61 (*gg*) O-6_2: −51 (*gg*) O-6_3: 176 (*tg*)	Res. 1: 116.3 Res. 2: 113.8 Res. 3: 115.9	34.2, 7.56	Res. 1: −26.3, −31.8 Res. 2: −26.7, −34.3 Res. 3: −25.7, −32.0	0.216

It also became clear that the unit-cell packing was tight and that there was only one probable arrangement of chains that was free of short, nonbonded contacts. In this arrangement, the favored chain-conformation was left-handed, as the right-handed conformation could not be refined to any packing position that was entirely free from short contacts. The refinement of the best left-handed structure also indicated that allowing the three 6-hydroxymethyl groups of one half-turn of the helix to rotate independently would improve packing. This variation would eliminate the 6-fold molecular symmetry, but would be consistent with the $P2_12_12_1$ crystal symmetry and the presence of the second-order meridional in the X-ray diagram. The best packing-structure thus obtained had O-6_1 and O-6_2 in the *gg* disposition, whereas O-6_3 was in the *tg* disposition. This arrangement allowed the formation of one weak, intramolecular, hydrogen bond per half-turn of the helix, between O-6 hydroxyl groups. The major features of this packing are shown in Table II and, as will be discussed later, the predicted structure is nearly identical with the structure obtained in the final X-ray refinement. No additional advantages in packing were realized by allowing the three residues of the half-turn to refine completely independently.

X-Ray refinement and location of KOH and H_2O. — The best packing-structure having no KOH units or H_2O molecules included gave an R''-factor of 0.523, with the observed intensities weighted at 1.0 and the unobserved at 0.5. (The weighted residual $R'' = \{[\sum w(|F_o| - |F_c|)^2/[\sum w|F_o|^2]\}^{1/2}$ was used in preference to the usual, unweighted R-factor as the refinement criterion, even though both residuals were always nearly identical in value). A difference-Fourier computed with the equatorial intensities, shown in Fig. 1, indicated a large area where K^+, OH^-, and the water molecules could be placed. The K^+ ion was placed in the structure first, at the highest density point in the difference Fourier, and its position was refined in projection, against the equatorial intensities. The OH^- ions and the water molecules (as oxygen atoms) were then added one at a time by the same procedure. With one K^+ ion and four oxygen atoms per asymmetric unit (three D-glucose residues) thus placed, the R''-factor for the equatorial intensities decreased to <0.10, and no further decrease in it was realized by adding more oxygen atoms. The z-coordinates of the ions and water molecules were then refined against three-dimensional data, one atom at a time, until no further improvement in the R''-factor occurred. In the final round of refinement, when the x,y,z coordinates of all water oxygen atoms and ions were allowed to vary simultaneously, stereochemical constraints were placed on the movement of these atoms in order to prevent the occurrence of short contacts. As done previously[12], this was accomplished by minimizing the function $\Phi = fR'' + (1 - f)Y$, where Y is the stereochemical packing-energy and the fraction f was optimally 0.985. As shown in Table II, the final R''-factor thus obtained was 0.328.

An alternative structure, containing two KOH units per asymmetric unit but no water, was also refined simultaneously with the foregoing structure. Its R''-factor reached a value of 0.332, but short contacts could not be eliminated and this structure was not considered further. Similarly, the best right-handed model was also refined, together with the best left-handed structure, despite the presence of short contacts

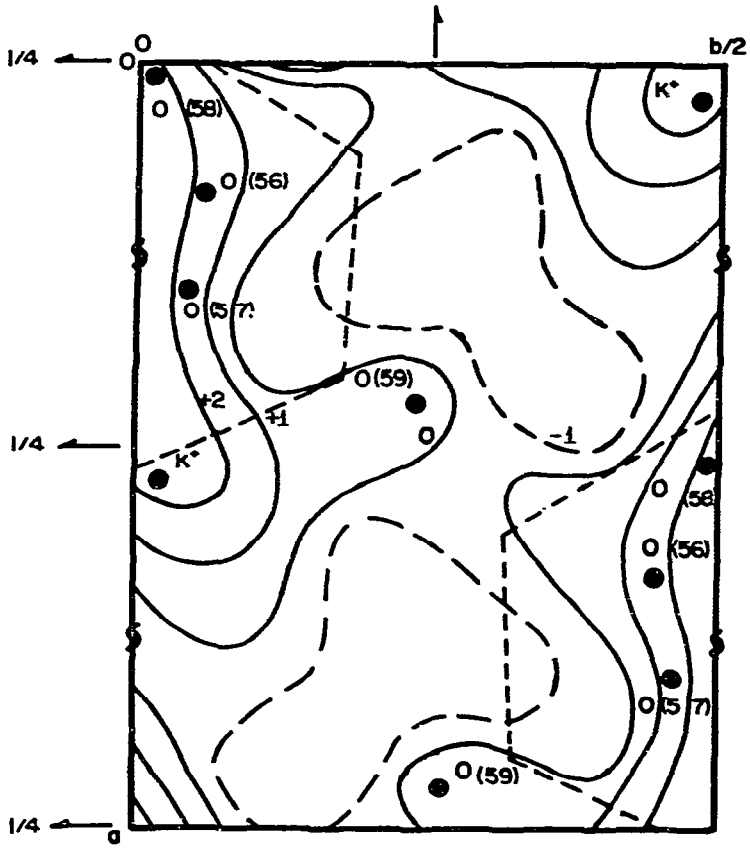

Fig. 1. Difference Fourier map (in projection down the *c* axis) calculated with the best packing 6_1 structure and all equatorial structure-amplitudes. The contour values are arbitrarily scaled. The outline of the chain is indicated by the virtual bonds (namely, vectors linking successive bridge oxygen atoms) in dashed lines and the final positions of the K^+ and oxygen atoms are shown.

in it. As it turned out, at every stage of refinement, the left-handed model was always superior to the right-handed one by a constant difference in the R''-factor of 0.08–0.09, and the right-handed model was eventually dropped from further refinement.

The best left-handed model at this stage of refinement still retained 6-fold molecular symmetry relative to the fixed components of the glucose rings, although the O-6 atoms were at independent positions. The optimum *VB* length was 4.50 Å. All attempts to refine with *VB* lengths other than this value resulted in increases in the R''-factor. At this point, the remaining 6-fold molecular symmetry was eliminated and the three residues of the asymmetric unit were allowed to refine independently. The parameters allowed to vary in this refinement included all three glycosidic bridge-angles, the ϕ,ψ angles for each residue, two torsion angles in each ring (rota-

tions about C-2–C-3 and C-5–O-5 bonds), and the O-6 rotations. Constrained refinement was again used by minimizing the function Φ.

This procedure resulted in a decrease of R'' to 0.270. No further refinement of individual residues was attempted, that is, all other bond-angles, torsion angles, and bond lengths were kept invariant. However, the addition of hydrogen atoms to the water oxygen atoms was simulated by using neon atomic-scattering factors for the water oxygen atoms, and an isotropic temperature-factor was included in the final round of refinement. This modification resulted in $R'' = 0.261$, with isotropic B = 4.66 Å^2. The major features of the final structure are shown in Table II. The calculated and observed structure-factors are listed in Table III (see footnote p. 000), and the atomic coordinates in Table IV. A stereo view of the structure is shown in Fig. 2. Hydrogen bonds, short contacts, and $K^+ \cdots O$ distances are listed in Table V.

TABLE IV

CARTESIAN ATOMIC COORDINATES OF ONE ASYMMETRIC UNIT OF THE FINAL STRUCTURE[a]

Atom[b]	x(Å)	y(Å)	z(Å)	*Atom*[b]	x(Å)	y(Å)	z(Å)
$C\text{-}1_1$	−1.528	−0.339	10.549	$H\text{-}1_2$	−0.038	3.825	14.853
$C\text{-}2_1$	−1.566	−1.860	10.512	$H\text{-}2_2$	−1.988	2.857	13.911
$C\text{-}3_1$	−0.384	−2.397	9.683	$H\text{-}3_2$	−0.586	0.227	13.954
$C\text{-}4_1$	−0.357	−1.686	8.325	$H\text{-}4_2$	−1.291	1.899	11.605
$C\text{-}5_1$	−0.361	−0.172	8.464	$H\text{-}5_2$	1.431	1.415	12.663
$C\text{-}6_1$	−0.468	0.525	7.134	$H\text{-}6A_2$	1.266	1.688	10.210
$O\text{-}2_1$	−1.531	−2.376	11.834	$H\text{-}6B_2$	2.194	2.881	11.001
$O\text{-}3_1$	−0.561	−3.810	9.519	$C\text{-}1_3$	3.766	3.247	17.978
$O\text{-}4_1$	0.801	−2.078	7.559	$C\text{-}2_3$	2.460	4.024	18.009
$O\text{-}5_1$	−1.517	0.199	9.235	$C\text{-}3_3$	1.380	3.297	17.187
$O\text{-}6_1$	−1.632	0.123	6.418	$C\text{-}4_3$	1.960	2.913	15.819
$H\text{-}1_1$	−2.398	−0.003	11.030	$C\text{-}5_3$	3.252	2.121	15.929
$H\text{-}2_1$	−2.462	−2.162	10.056	$C\text{-}6_3$	3.858	1.847	14.578
$H\text{-}3_1$	0.515	−2.213	10.194	$O\text{-}2_3$	2.012	4.253	19.338
$H\text{-}4_1$	−1.215	−1.968	7.790	$O\text{-}3_3$	0.262	4.180	17.039
$H\text{-}5_1$	0.511	0.140	8.959	$O\text{-}4_3$	1.018	2.132	15.058
$H\text{-}6A_1$	0.386	0.317	6.560	$O\text{-}5_3$	4.175	2.954	16.650
$H\text{-}6B_1$	−0.516	1.560	7.301	$O\text{-}6_3$	3.056	0.952	13.809
$C\text{-}1_2$	0.076	2.913	14.343	$H\text{-}1_3$	4.508	3.844	18.421
$C\text{-}2_2$	−1.275	2.210	14.334	$H\text{-}2_3$	2.630	4.958	17.562
$C\text{-}3_2$	−1.198	0.934	13.476	$H\text{-}3_3$	1.077	2.430	17.696
$C\text{-}4_2$	−0.606	1.281	12.106	$H\text{-}4_3$	2.161	3.796	15.288
$C\text{-}5_2$	0.712	2.031	12.210	$H\text{-}5_3$	3.088	1.222	16.447
$C\text{-}6_2$	1.224	2.500	10.874	$H\text{-}6A_3$	4.818	1.441	14.701
$O\text{-}2_2$	−1.670	1.902	15.663	$H\text{-}6B_3$	3.941	2.755	14.057
$O\text{-}3_2$	−2.524	0.406	13.343	K^+	4.898	0.756	7.846
$O\text{-}4_2$	−0.413	0.095	11.308	O-56	1.593	0.655	−1.351
$O\text{-}5_2$	0.505	3.201	13.020	O-57	1.812	−0.404	16.947
$O\text{-}6_2$	0.400	3.525	10.320	O-58	0.075	0.233	0.683
				O-59	−0.423	3.278	19.641

[a]Origin of the coordinate system is half-way between three pairs of non-intersecting screw axes (as shown in Fig. 3). [b]Subscript indicates residue number.

TABLE V

INTERMOLECULAR HYDROGEN BONDS, SHORT CONTACTS, AND K^+—O DISTANCES IN THE FINAL MODEL

Hydrogen bond and distance (Å)[a]		*Short contact and distance (Å)*[b]		*K+—O Distance (Å)*	
O-2_1—O-2_3[c]	2.53	C-1_2—K^{+d}	2.53	O-5_2—K^{+d}	2.68
O-3_2—O-2_3[c]	2.54	O-6_1—H-2_3[d]	1.91	O-3_3—K^{+d}	2.77
O-3_2—O-6_2[c]	2.96	H-1_2—K^{+d}	1.68	O-6_1—K^{+e}	2.79
C-6_2—O-6_3[d]	2.98	H-4_2—H-$6B_2$[d]	1.68	O-5_1—K^{+e}	2.85
O-2_3—O-59[e]	2.64			K^+—O-57[f]	3.13
O-3_3—O-59[e]	2.84			K^+—O-56[f]	3.21
O-6_3—O-58[f]	2.60				
O-3_2—O-58[f]	2.53				
O-3_1—O-58[e]	2.39				
O-6_2—O-58[e]	2.91				
O-2_1—O-59[e]	2.94				
O-3_3—O-57[e]	2.63				
O-56—O-58[e]	2.57				

[a]Atom subscripts indicate residue number. [b]Contacts shorter than the minimum distances quoted in ref. 14. [c]Symmetry −x,1/2+y,1/2−z. [d]Symmetry 1/2+x,1/2−y,−z. [e]Symmetry x,y,z. [f]Symmetry 1/2−x,−y,1/2+z.

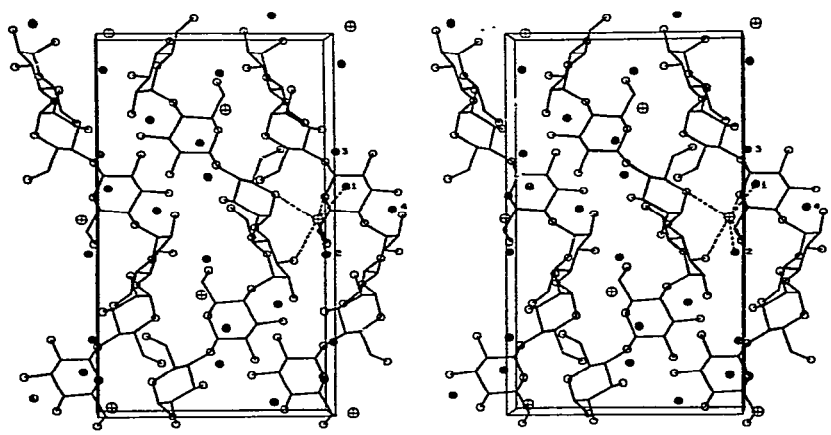

Fig. 2. Stereo view of the final structure. The center chain and two corner chains of the unit cell are shown, with all hydrogen atoms omitted for clarity. The coordination of the K^+ ion (represented by the symbol ⊕) is shown by dashed lines and all non-amylose oxygen atoms are denoted by filled circles. The numbers 1–4 indicate, respectively, oxygen atoms O-56 to O-59.

DISCUSSION

The ϕ,ψ-map of amylose, calculated with a residue length VB = 4.50 Å and the glycosidic bridge-angle at 115°, is shown in Fig. 3. It is clear that the chain conformation of the potassium hydroxide–amylose complex does not coincide with any of the minima in the map. This result is because of the absence of regular, intra-

molecular hydrogen bonds in the structure. However, this apparent lack of stabilization is evidently compensated for by the presence of intermolecular hydrogen-bonds and ionic interactions. The latter are undoubtedly the main reason why the chain is in a more-extended conformation than any of the other amylose polymorphs. Once again, this result illustrates the point that the conformational energy alone may not be a sufficiently accurate indicator of the actual conformation found in the crystal structure.

The interactions of the K^+ ion with oxygen atoms is shown in Fig. 2. The K^+ ion is surrounded by six oxygen atoms, four from the amylose chain and two from water molecules, at distances ranging from 2.68 to 3.21 Å (compare Table V). It is difficult to judge which of the oxygen atoms constitutes the OH^- ion, but O-58 may be a likely candidate, because it is only 2.39 Å from the O-3 hydroxyl group and 2.77 Å from the K^+ ion, and it is at hydrogen-bond distances to four other hydroxyl groups

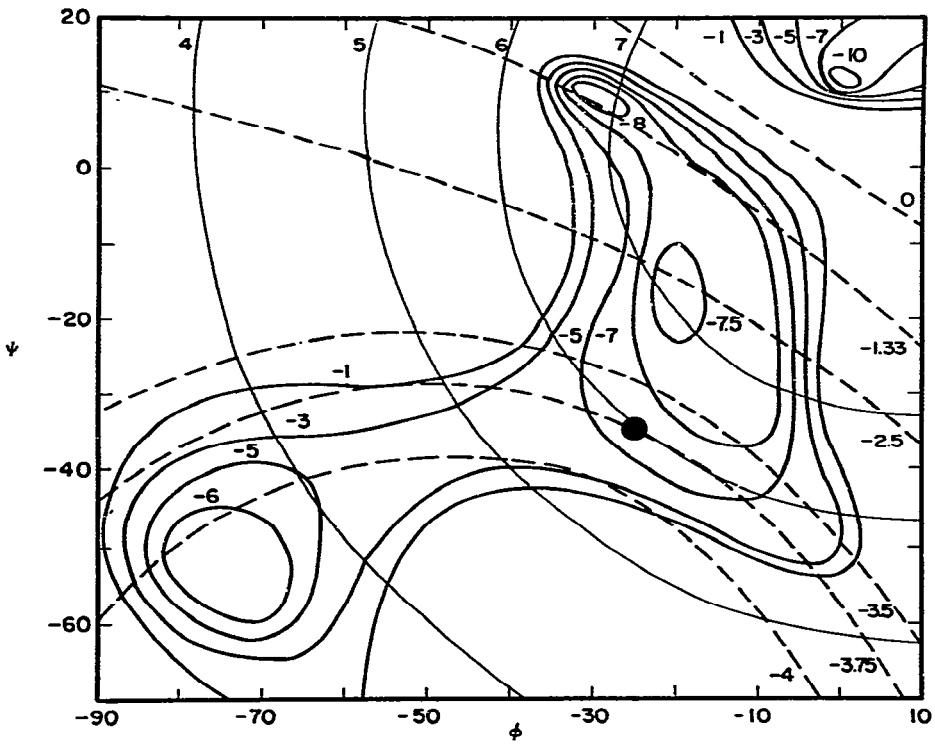

Fig. 3. A section of the ϕ,ψ-map of amylose calculated with a $VB = 4.50$ Å residue and a glycosidic bridge-angle of 115°. Only the region containing left-handed conformations is shown. The bold contours indicate nonbonded conformational-energy in kcal/mol, the thin lines indicate the number of residues per turn, n, and the dashed lines denote the axial rise per residue, h, in Å. Negative values of h correspond to left-handed helices. The conformation of the final structure is indicated by a filled circle. The conventions used in the calculation of the map are those of ref. 13. The ϕ,ψ scales are in degrees.

or water molecules (compare Table V). As shown in Table V, nearly all of the amylose as well as non-amylose oxygen atoms form hydrogen bonds, but only half of the oxygen atoms are either part of a hydrogen-bond sequence or are fully 4-coordinated in the case of the water molecules. This feature results in three fairly extensive, although incomplete, hydrogen-bond chains, namely: $O\text{-}59^{c}$–$O\text{-}2_1$–$O\text{-}2_3^{c}$–$O\text{-}3_2$–$O\text{-}58^{f}$–$O\text{-}6_3$, with $O\text{-}6_2^{c}$ branching from $O\text{-}2_3^{c}$; $O\text{-}3_1$–$O\text{-}58^{c}$–$O\text{-}6_2$–$O\text{-}6_3^{d}$, with $O\text{-}56^{c}$ branching from $O\text{-}58^{c}$; and $O\text{-}2_3$–$O\text{-}59$–$O\text{-}3_3$–$O\text{-}57^{c}$. The structure is thus reasonably well hydrogen-bonded, but not strongly stabilized. This factor accounts for the ready water solubility, in contrast to the highly hydrogen-bonded, double-stranded structures of A- and B-amylose[5,6].

The reasonably low R''-factor and the coincidence of the final structure with the predicted best packing-structure (compare Table II) both indicate that the structure is undoubtedly correct in its major features. However, as comparison of the observed and calculated structure-factors of inidividual reflections shows, there are some disagreements that indicate that the structure may not be completely correct in all details. In particular, the final structure predicts only a weak, second-order meridional, whereas the observed meridional is of medium intensity. The intensities of the predicted and observed fourth-order meridionals are in good agreement. The meridional reflections were not used in the refinement, but it was noted that the intensity of the second-order meridional was most sensitive to the distortions in the asymmetric unit, namely, deviations from 6-fold molecular symmetry. The lack of better agreement between the predicted and observed, second meridional intensities thus suggests that the true structure may be more distorted than is at present indicated. By refining all bond lengths, bond angles, and torsion angles of the three-residue asymmetric unit, better agreement in intensities could undoubtedly be reached, but the wisdom of attempting refinement of so many variables against 99 intensities is questionable.

The K^+ and OH^- ions and the water molecules are very probably in correct positions, both because of the coordination of K^+ with the oxygen atoms, and because it became apparent during the refinement that there was very little choice where to place these ions and molecules in the unit cell.

With the determination of this crystal structure, the features of three amyloses in the sequence leading from amylose triacetate to V-amylose are now known with reasonable accuracy[3,4,8]. All three structures are based on left-handed chain conformations, which is consistent with the observation that both conversions, namely from amylose triacetate to potassium hydroxide–amylose and from the latter to V-amylose, proceed readily and without passing through completely amorphous, intermediate stages. In contrast, conversion of potassium hydroxide–amylose to the right-handed A- and B-amyloses proceeds slowly and exhibits a prolonged, amorphous period during the process[5,6].

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