

Polymorphism of Amylose V Complexes

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Lamellar crystals of amylose V complexes with the 6_1 , 7_1 and 8_1 helical configurations can be prepared from aqueous solutions of amylose by using various complexing agents. It is noted that the crystal shape can be explained by the symmetry of the unit cell in the basal plane and the chain packing in the unit cell is concerned with the symmetry of helical chains. The existence of 6_1 , 7_1 and 8_1 helices which occurs stepwise with the number of glucopyranose an integer is discussed.

§ 1. Introduction

Amylose has the interesting property of forming crystalline complexes with low molecular weight substances, such as iodine, alcohols, ketones and fatty acids. Studies on the properties and structures of these complexes were carried out extensively by Bear¹⁾ and Rundle²⁾⁻³⁾ in the 1940's. Thereafter only little attention has been directed to the structural study of amylose V complexes. In part, this may be accounted for by difficulties encountered in producing oriented specimens of amylose V crystals and by limitations on structural information obtainable from x-ray and other physical techniques applied to powder specimens.

In recent years, the morphology and structure of single crystals of amylose V complexes by precipitation from aqueous solution with various complexing agents have been reported⁴⁾⁻⁵⁾ from our laboratory. In these papers it was shown that the crystals consisted of lamellae about 100 Å in the thickness and had many features in common with lamellar crystals from linear synthetic polymers. Thus it was confirmed that the concept of chain folding could be applied to lamellar crystals of amylose V complexes with large helix diameter. The electron diffraction study for single crystals of the *n*-butyl alcohol complex provided strong support for the helical structure pictured by Rundle³⁾ as closed packed helices with six glucose residues per turn (6_1 helix). Furthermore, it was found for the first time that crystals of amylose V complexes with 7_1 and 8_1 helical configurations can be obtained

by using branched chain alcohols (*iso*-propyl, *iso*-butyl, *sec*-butyl and *tert*-butyl alcohols) and α -naphthol, respectively.

The purpose of the present paper is to review our results of morphological and structural studies on single crystals of amylose V complexes and to discuss the polymorphism of amylose V complexes.

§ 2. Experimental

Preparation of Single Crystals of Amylose V Complexes

We used methyl alcohol, ethyl alcohol, *n*-propyl alcohol, *n*-butyl alcohol, cetyl alcohol, stearic acid and oleic acid as complexing agents for crystals with 6_1 helical configuration, *iso*-propyl alcohol, *iso*-butyl alcohol, *sec*-butyl alcohol, *tert*-butyl alcohol and cyclohexanol for 7_1 helical configuration and α -naphthol for 8_1 helical configuration. Single crystals of amylose V complexes with these complexing agents were prepared as follow. A 10 g portion of potato starch was dispersed in 800 ml of water by heating the mixture at 70°C for 1 hr. in a thermostat. The hot turbid starch dispersion was filtered rapidly. The resulting amylose solution was heated to 95°C again, the complexing agents were added and the whole was stirred gently. Crystallization was performed at constant temperature between 40°C and 70°C, and then cooled slowly to room temperature. The resulting crystals were examined by electron microscopy, and x-ray and electron diffraction. To obtain the degree of polymerization of the leached potato amylose, the intrinsic viscosity in 1N NaOH at

22.5°C was measured. This value was 115 ml/g and corresponds to an average degree of polymerization of 850 from the viscosity-molecular weight relation derived by Greenwood et al⁹.

Morphological Observations and Electron Diffraction Studies

Drops of the above crystal suspension were placed on carbon coated grids and the solvents were evaporated. The specimens were shadowed with Pt-Pd and examined by direct transmission in a HU 11B electron microscope. Selected area electron diffraction experiments were carried out in the same instrument. Calibration of the diffraction spots was made with the aid of a thin evaporated layer of aluminum on the same specimens.

X-ray Diffraction Studies

The film was obtained by filtering the crystal suspension carefully. X-ray diffraction photographs for the oriented films of the sedimented lamellar crystals were obtained in a cylindrical camera with the use of nickel-filtered $\text{CuK}\alpha$ radiation.

§ 3. Results and Discussion

Crystal Habits of Single Crystals of Amylose V Complexes

Figures 1, 2, 3 and 4 show electron micrographs of the typical crystals of the cetyl alcohol, *n*-butyl alcohol, *iso*-propyl alcohol and

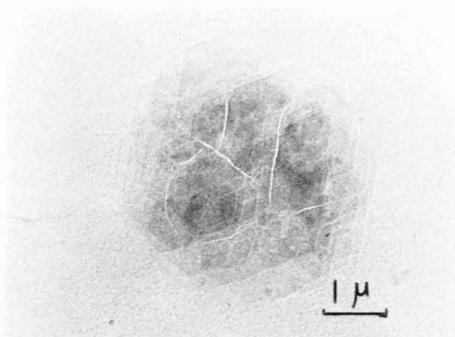


Fig. 1 Electron micrograph of crystals of the cetyl alcohol complexes. The crystals favor growth with dislocation centered spiral structure.

α -naphthol complexes. These micrographs show that the crystals consist of stacks of thin lamellae whose thickness is estimated to be about 100 Å from shadow length measurements. The shape of lamellar crystals depends on the complexing agents. The results of our

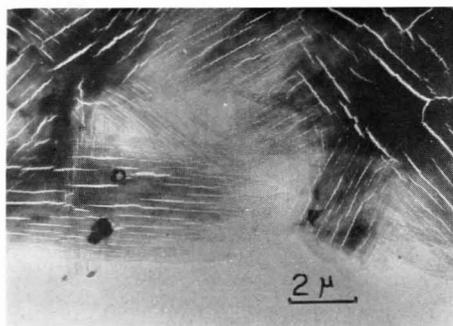


Fig. 2 Electron micrograph of crystals of the *n*-butyl alcohol complexes. These crystals show cracks along the direction of the longer side of the rectangular lamellae.



Fig. 3 Electron micrograph of crystals of the *iso*-propyl alcohol complexes.

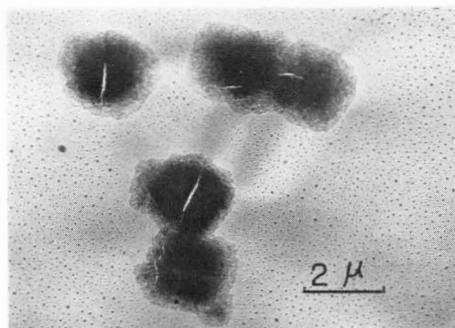


Fig. 4 Electron micrograph of crystals of the α -naphthol complexes.

morphological investigations are presented in Table 1. It will be noted that all of the crystal shape except the *n*-butyl and *n*-propyl alcohol complexes can be explained by the symmetry of the unit cell in the basal plane obtained from the electron diffraction pattern. That is, the crystals are hexagonal, rectangular and square-shaped lamellae according to the hexagonal, orthorhombic and tetragonal sym-

Table 1. Crystal habits of amylose V complexes with various complexing agents

Complexing agent	Crystal habit	Symmetry of the electron diffraction pattern
methyl alcohol ethyl alcohol cetyl alcohol stearic acid oleic acid	hexagon	hexagonal
<i>n</i> -butyl alcohol <i>n</i> -propyl alcohol	rectangle	hexagonal
<i>iso</i> -propyl alcohol <i>iso</i> -butyl alcohol <i>sec</i> -butyl alcohol <i>tert</i> -butyl alcohol cyclohexanol	rectangle	orthorhombic
α -naphthol	square	tetragonal

metry of the electron diffraction patterns. The rectangular habit in the *n*-butyl and *n*-propyl alcohol complexes are consistent with the fact that the crystal structure has orthorhombic symmetry rather than hexagonal at the time when the lamellar crystals are formed in the supercooled solution as mentioned in

the previous paper⁶⁾. The occurrence of this orthorhombic structure in the crystals with 6_1 helical configuration is presumably due to an anisotropic hydrated structure. Electron diffraction studies did not show the unit cell for the hydrated crystals, because this state of the crystals was unstable under the microscope vacuum.

Three Crystal Polymorphs with Different Helical Configurations in Amylose V Complexes

Selected area electron diffraction patterns from the lamellar crystals obtained with *n*-butyl alcohol, *iso*-propyl alcohol and α -naphthol are shown in Figure 5. X-ray diffraction photographs for the oriented films of the sedimented lamellar crystals are shown in Figure 6. It has been suggested in our previous papers⁶⁾⁻⁸⁾ that the crystals of each complex have 6_1 , 7_1 and 8_1 helical configurations from the studies of the above electron and x-ray diffraction patterns. The results of our structural investigations are summarized in Table 2. It is especially interesting to note that the chain packing in the unit cell is concerned with the symmetry of helical chains. Since the 6_1 and 8_1 helical chains have a six-fold screw

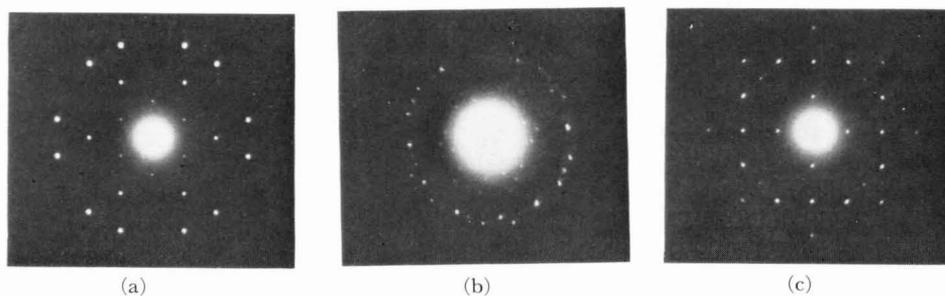


Fig. 5 Selected area electron diffraction patterns from the lamellar crystals obtained with (a) *n*-butyl alcohol, (b) *iso*-propyl alcohol and (c) α -naphthol.

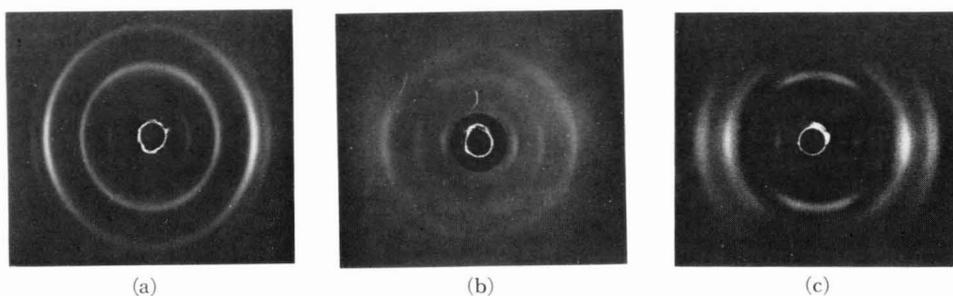


Fig. 6 X-ray diffraction photographs for the oriented films of the sedimented lamellar crystals obtained with (a) cetyl alcohol, (b) *iso*-propyl alcohol and (c) α -naphthol.

Table 2. The structural data of amylose V complexes

Complexing agent	Crystal system	Lattice constants	Helical configuration
methyl alcohol ethyl alcohol <i>n</i> -propyl alcohol <i>n</i> -butyl alcohol cetyl alcohol stearic acid oleic acid	hexagonal	$a=26.4\text{\AA}$ $c=7.8\text{\AA}$	6_1
<i>iso</i> -propyl alcohol <i>iso</i> -butyl alcohol <i>sec</i> -butyl alcohol <i>tert</i> -butyl alcohol cyclohexanol	orthorhombic	$a=30.0\text{\AA}$ $b=28.3\text{\AA}$ $c=7.8\text{\AA}$	7_1
α -naphthol	tetragonal	$a=b=22.9\text{\AA}$ $c=7.8\text{\AA}$	8_1

axis and an eight-fold screw axis, respectively, the over-all structure of each complex shows hexagonal and tetragonal features. On the other hand, the crystals with 7_1 helical configuration have intermediate features of above complexes and are orthorhombic in crystal structure, since a seven-fold screw axis of chain can not be realized in the over-all crystal structure.

The results in Table 2 demonstrate the determinant role played by the organic complexing agents in affecting the helical configuration of amylose molecules in crystals. The size of the cavity in the interior of 6_1 , 7_1 and 8_1 helices is 4.8\AA , 6.3\AA and 7.8\AA , respectively, from the geometrical considerations as shown in the previous paper³⁾ and is proper to accommodate the complexing agents. The detailed nature of the interaction between amylose and the complexing agents is not clear at present. However, it is evident that random coil molecules of amylose are converted to helical molecules having the complexing agents in the cavity when the crystallization of amylose from aqueous solution occurs.

Then, we consider the existence of 6_1 , 7_1 and 8_1 helices which occurs stepwise with the number of glucopyranose. We have confirmed¹⁰ that each form of three crystal polymorphs with different helical configurations is stable even on annealing at 250°C , although transitions between the forms occur special conditions^{7),11)}.

It is now established both theoretically and experimentally that the glucopyranose residues in amylose exist only in the C1 conformation¹²⁾. Since there is rotational about the ether linkage $C_1-O-C'_4$, the helical configuration of the amylose chain is determined principally by a set of internal rotation angles $\tau_{H_1-C_1-O-C'_4}$, $\tau_{C_1-O-C'_4-H'_1}$ (in which the atoms C_1 , H_1 belong to the first residue and C'_4 , H'_1 to the second residue.) which is the same at all ether linkages. However, various sets of internal rotation angles can be considered even if the identity period is determined. Hence, the possible conformations are limited by the minimum conformational energy. The non-bonded energy (van der Waals) has been computed for isolated helical amylose chains as a function of the internal rotation angles by Sundararajan and Rao¹²⁾. Their results do not indicate that integral helices are preferred. On the other hand, the existence of 6_1 , 7_1 and 8_1 helices in the molecular configuration of amylose V show that variation of the helix diameter occurs stepwise with the number of glucopyranose residues an integer. Therefore, the relative position of residues between one turn and the next can be expected to be similar. It may be speculated that the hydrogen bonding forces operating between successive turns play an important role in determining the possible configuration of V amylose.

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