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# The crystalline domains in potato starch granules are arranged in a helical fashion

**Gerrit T. Oostergetel & Ernst F.J. van Bruggen**

*Bioson Research Institute, University of Groningen, Nijenborgh 16, NL-9747 AG Groningen, The Netherlands*

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The structural basis for the physical properties of starches from different botanical sources is still poorly understood. Particularly at the level of the crystalline domains the present knowledge concerning the structure of starch is limited. This paper reports the semi-crystalline structure of potato starch granules by electron optical tomography and by cryo electron diffraction. It is concluded that the crystalline domains in the amylopectin form a continuous network of left-handed helices, which appears as a well-ordered skeleton for the starch granule. This type of super-helical lamellar structure has not been reported before, either in natural or in synthetic polymers.

## INTRODUCTION

Starch, a carbohydrate reserve material in green plants, contains amylose and amylopectin as its major components. Amylose is an essentially linear polymer consisting of $\alpha$-(1 → 4) linked glucose residues, whereas amylopectin is highly branched via additional $\alpha$-(1 → 6) linkages. The branch points in the amylopectin molecules are not randomly distributed along the molecule, but are clustered and the interjacent linear segments form thin (~ 5 nm) crystalline lamellar domains. These domains are visible in transmission electron micrographs of starch granule thin sections (Kassenbeck, 1975, 1978) or fragments (Yamaguchi *et al.*, 1979; Oostergetel & van Bruggen, 1989) with a regular spacing of ~ 10 nm (Blanshard *et al.*, 1984; Oostergetel & van Bruggen, 1989) and appear in two main directions with a relative angle of ~25°. The 'cluster model' for the amylopectin molecule describing the alternating amorphous and crystalline layers in starch is now generally accepted (Zobel, 1988; Manners, 1989) but does not explain why the crystalline lamellae are seen locally in essentially two different orientations, how these lamellae are arranged spatially and how the amylopectin molecules fit into this lamellar structure.

Analysis of X-ray fibre diffraction data from amylose fibres and from powder diffractograms of native starch has led to atomic models for the crystalline structures in both the A and B conformations (Imberty & Perez, 1988; Imberty *et al.*, 1988), found in cereal starches and tuber starches, respectively. However, ~ 70% of the starch granule is amorphous. So, even though detailed information of part of the structure is known at the atomic level, knowledge of the structure at the level of crystalline and amorphous domains, which is very important for an understanding of the physical properties of starch, is still lacking.

The apparent similarity of the semi-crystalline structure in starch granules with that seen in synthetic polymers like polyethylene (Bassett, 1978) raises the question as to what extent the two structures are actually similar when compared in three dimensions rather than at the level of two-dimensional projections.

It has been shown previously (Oostergetel & van Bruggen, 1989) that the domain structure in starch can be studied well by transmission electron microscopy of small, negatively stained granule fragments. Under the proper preparation conditions the lamellar structure is not disrupted as was concluded from comparison of results from electron microscopy with those from small-angle X-ray diffraction on starch suspensions.

The aim of this study was to arrive at a model for the structural organisation of amylopectin in starch granules. This was achieved by combining electron tomography on negatively stained potato starch granule fragments and cryo electron diffraction of frozen-hydrated (unstained) granule fragments.

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## MATERIALS AND METHODS

### Starch

Starch from potato was isolated as described by Vos-Scheperkeuter *et al.* (1986). Partial hydrolysis was carried out in 2·2 N HCl at 35°C for 16 h. The hydrolysed starch was washed with deionised water until the pH was $\geqslant 5$.

### Electron microscopy and electron diffraction

Fragments of mildly hydrolysed potato starch granules and specimens for transmission electron microscopy were prepared as described previously (Oostergetel & van Bruggen, 1989). Electron micrographs were recorded on Kodak SO-163 film in a JEOL 1200EX transmission electron microscope equipped with an EM-SRH10 specimen rotation holder, at a magnification of 27 000×. Single-axis tilt series were recorded with tilt angles from −60° to +60° relative to the electron beam with an increment of 3° or 6°. For electron diffraction fragments were adsorbed to a carbon support film from a suspension in water. The specimen was equilibrated overnight in a desiccator at a relative humidity of 85% at 20°C, fast frozen in liquid nitrogen and transferred under liquid nitrogen to a Philips PW 6591 cooling holder (Chanzy *et al.*, 1977). Electron diffractograms were recorded in a Philips EM400 electron microscope at 120 kV on Kodak DEF-5 X-ray film using a camera length of 1300 mm and a specimen temperature of −170°C.

### Tomographic reconstruction

Negatives from tilt series were digitised on a Joyce-Loebl Scandig 3 rotating-drum densitometer using a 25 × 25 $\mu$m sampling aperture and sampling grid, corresponding to a pixel size of 0·93 nm at the specimen level.

From the digitised images the tilt angle, the direction of the tilt axis and the translational and rotational alignment parameters were determined using reference points in the background. The densities were corrected for fluctuations in the intensity of the primary electron beam. Three-dimensional reconstructions were carried out using an iterative tomographic reconstruction method (GSIRT) (Lakshminarayanan & Lent, 1979) implemented in the IMAGIC image processing system (van Heel & Keegstra, 1981) on a CONVEX C1-XP computer. The reconstructed volume was low-pass Fourier-filtered to a resolution of 5 nm. The effect of radiation damage was assessed by comparing the first (60°) micrograph in a tilt series with one, recorded after the tilt series was completed, using a Fourier ring correlation criterion (van Heel, 1987).

## RESULTS AND DISCUSSION

### Three-dimensional reconstruction

In electron micrographs of negatively stained starch granule fragments the lamellar structure from the amylopectin is clearly visible (Fig. 1). Most fragments

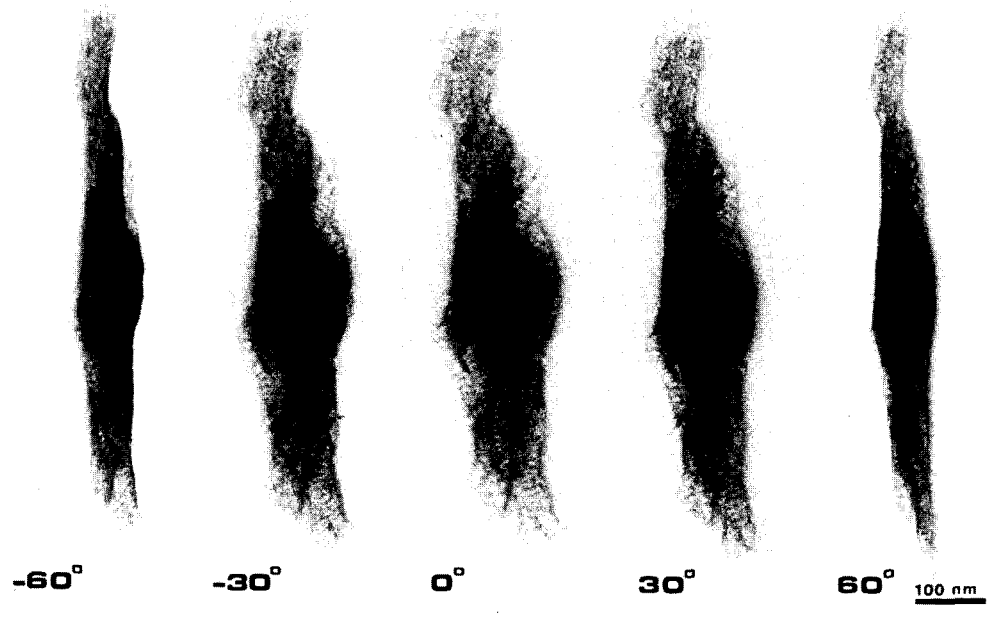


**Fig. 1.** Five projections from a tilt series of a potato starch granule fragment, negatively stained with 1% uranyl acetate. The tilt axis is vertical; tilt angles were varied from −60° to +60° with a 3° increment.

are elongated with the lamellae slightly tilted relative to the long axis of the fragment. When the fragment is tilted in the microscope, the projected lamellar structure changes but the interpretation in terms of the three-dimensional arrangement of the crystalline lamellae is not straightforward. Therefore, the three-dimensional structure of several fragments was calculated using a tomographic reconstruction procedure from series of projections obtained by tilting the specimen in the microscope from −60° to +60° with an increment of 3° or 6°. Figure 1 shows five projections out of a series of 41 from a potato starch granule fragment, ~800 nm long and 150 nm wide. After the reconstruction the resulting densities were three-dimensionally filtered to remove uninterpretable details smaller than 5 nm. Comparison by Fourier ring correlation (van Heel, 1987) of the first micrograph in a tilt series with one recorded after the tilt series had been completed, indicates that details larger than 5 nm are not significantly affected by electron radiation damage. The resolution in the vertical direction (perpendicular to the plane of the support film) is estimated to be ~8 nm. Slices from the reconstructed volume of 864 × 216 × 216 pixels were printed on glass plates which were then stacked to form a spatial representation of the density (not shown). Details of the reconstruction, including a number of model calculations, will be published elsewhere. Figure 2 shows a selection of slices through the reconstruction from the fragment in Fig. 1, ~9·3 nm apart and parallel to the plane of the untilted specimen. It indicates a total thickness of ~80 nm for the fragment. This reconstruction from the fragment of Fig. 1 and a second example is shown as stereographic surface representations (van Heel, 1983) in Fig. 3. From the presented reconstructions it is evident that the crystalline lamellae are tilted relative to the long axis of the fragments. More surprisingly though, as can be seen clearly in the detail shown in Fig. 3C, the crystalline lamellae form short helical stretches. Closer inspection of the reconstructed granule fragments reveals that the complete semi-crystalline structure is built up from more or less continuous left-handed helical segments, with a diameter of ~18 nm and a pitch of 10 nm.

**Electron diffraction**

The helical arrangement of the crystalline lamellae raises the question as to how the crystal lattices are orientated and how one could envisage the branched amylopectin molecules be arranged to form a large helical structure. In order to answer the question of the orientation electron diffraction was carried out on starch granule fragments in the frozen hydrated state (Chanzy *et al.*, 1977). This technique can provide information about the local structure from an area as small as in the order of 0·1 μm across, contrary to X-ray diffraction where samples several orders of magnitude larger are required. An electron diffractogram of a frozen hydrated starch granule fragment, recorded at −170°C is presented in Fig. 4. This diffractogram, which shows reflections corresponding with a resolution of 0·2 nm, is similar to the B-type X-ray diffractogram from amylose fibres (Wu & Sarko, 1978) for which a hexagonal unit cell with parallel left-handed double helices has been proposed (Imberty & Perez, 1988). It indicates that locally in the starch granule fragment the crystallites are all orientated with the crystallographic c-axis approximately parallel to the axis of the large helix, despite the different orientations of the lamellar planes.

**Packing of the helices**

Sections from the reconstructions perpendicular to the helical axis show a more or less regular packing of the helices (see Fig. 5 for an example). Assuming that a close interaction between the crystalline lamellae in

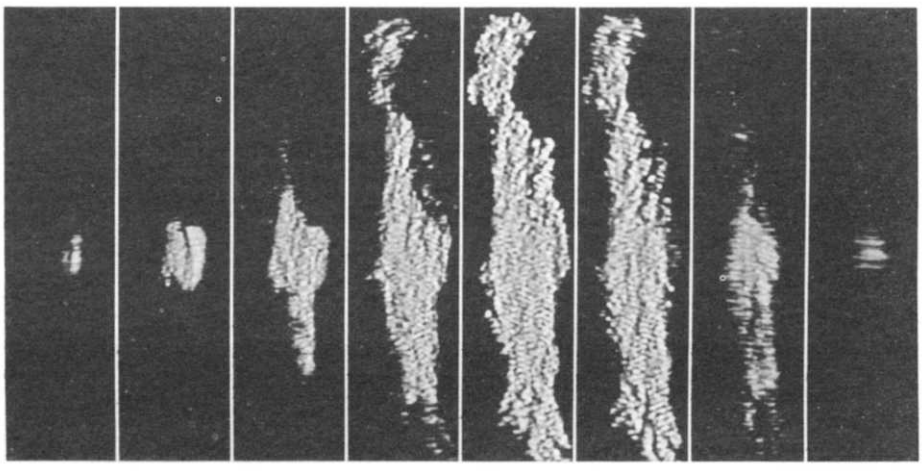

**Fig. 2.** Horizontal slices (thickness 0·93 nm) through the reconstruction from the projections in Fig. 1. Every tenth slice is shown.

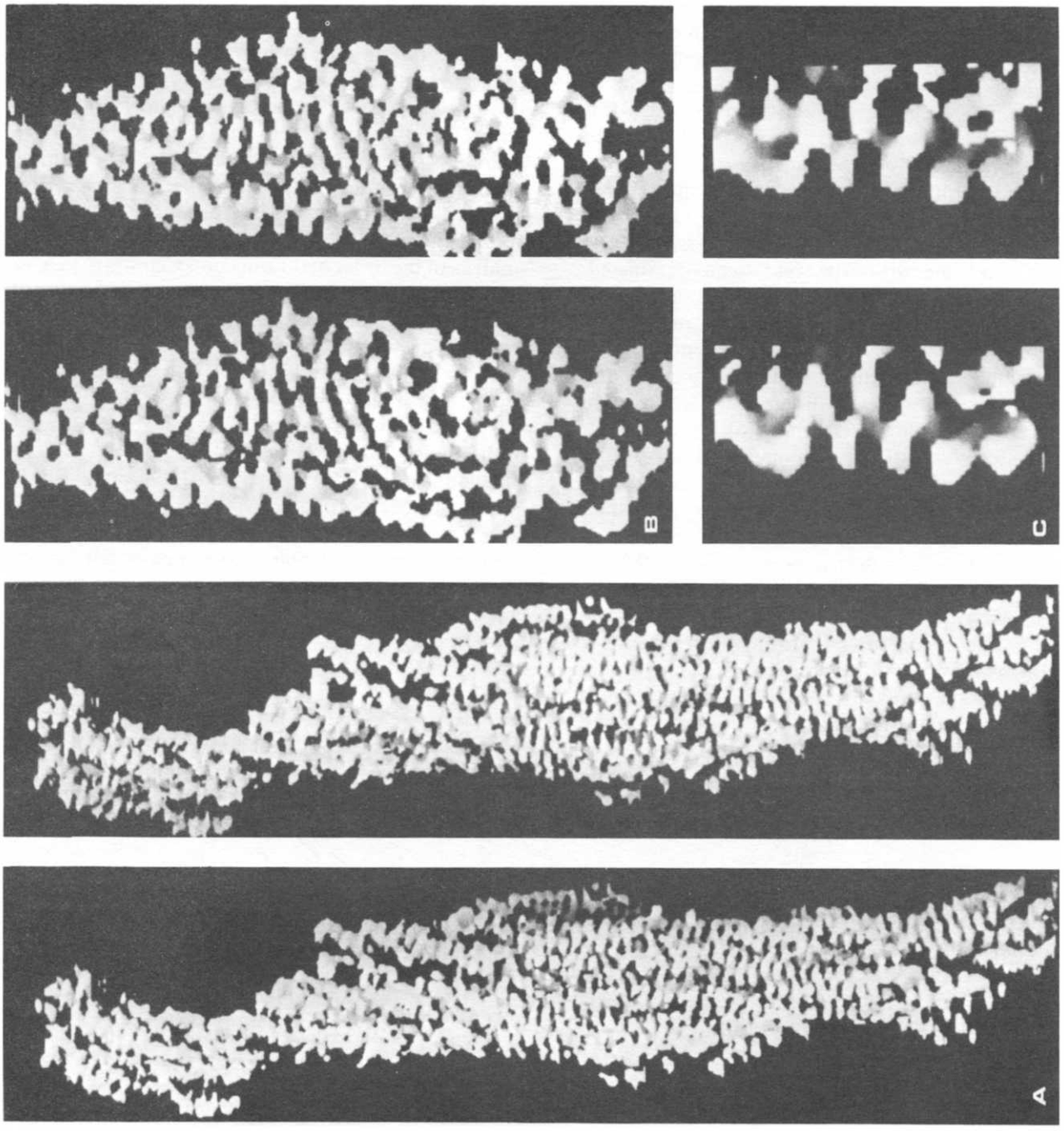


**Fig. 3.** Stereo images of two three-dimensional reconstructions of starch granule fragments in a surface representation (A and B); C is a detail from B in which the helical organisation is clearly visible. White is high density, i.e. crystalline, stain excluding material.

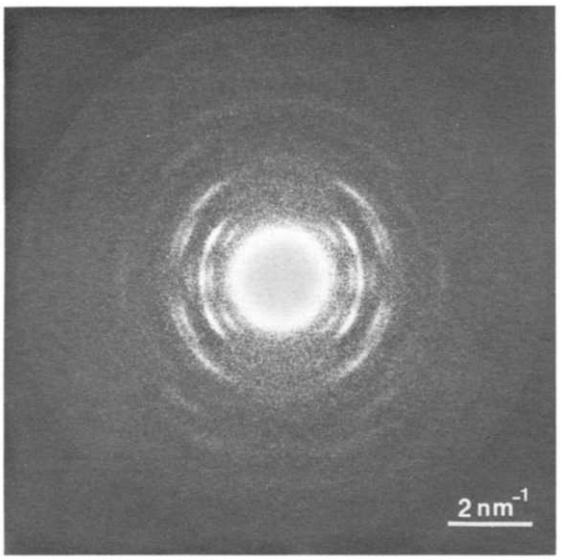


**Fig. 4.** Electron diffraction pattern of a frozen hydrated starch granule fragment similar to that shown in Fig. 1. The direction corresponding to the long axis of the fragment is vertical.

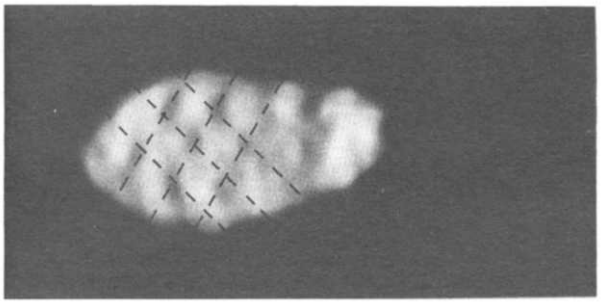

**Fig. 5.** Central section of the reconstruction shown in Fig. 3A, perpendicular to the long axis, showing the packing of the helices: the central holes in the helices are black. The roughly tetragonal packing is indicated by dashed lines.

neighbouring helices is necessary to provide the long range regularity in the structure seen in the electron micrographs, a tetragonal arrangement of the helices is most likely, even though the cross-sectional views suggest that a hexagonal packing might be possible. The observation that similar projections are seen in four different orientations when the reconstructed fragment is rotated over 360° around the long axis supports a tetragonal arrangement. A hexagonal packing is not compatible with the observed tetragonal symmetry.

## Model for the domain structure of amylopectin

Combining the data from three-dimensional reconstructions and the electron diffraction experiments leads us to propose the model in Fig. 6B for the semi-crystalline structure and the arrangement of amylopectin

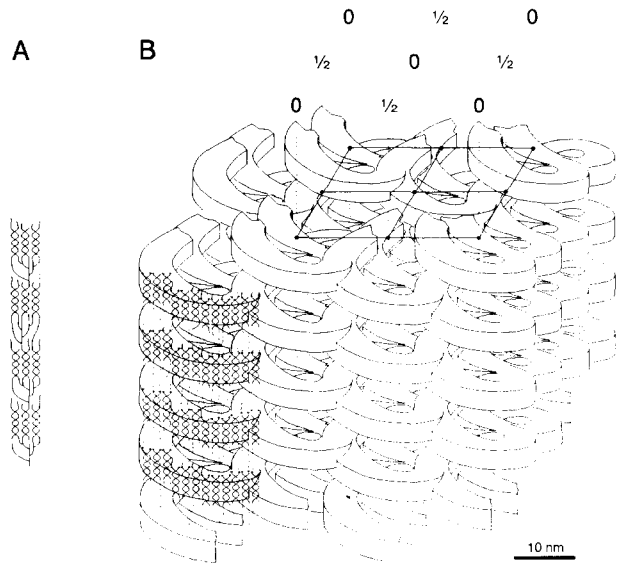


**Fig. 6.** Schematic model for the arrangement of amylopectin in potato starch. (A) Model for the amylopectin molecule, showing the clustering of the $\alpha$-(1→4),$\alpha$-(1→6) branch points, and the double helical linear $\alpha$-(1→4) glucan chains. The layers containing the branch points are amorphous, whereas the linear segments are crystallized to form 5 nm thick crystalline lamellae. (B) The crystalline layers containing the double helical linear segments in the amylopectin molecules form a continuous network consisting of left-handed helices packed in a tetragonal array. Neighbouring helices are shifted relative to each other by half the helical pitch (indicated by 0 and $\frac{1}{2}$). Four amylopectin molecules (A) are projected into one of the helices.

molecules (Fig. 6A) in potato starch. In this model the helices form a continuous, regular crystalline network, which appears as a skeleton around which the rest of the starch granule is built. The linear segments of approximately 5 nm long form double helices which are crystallised into 5 nm thick lamellae, alternating with amorphous layers in which the $\alpha$-(1→4), $\alpha$-(1→6) branch points are located. Since neighbouring helices interpenetrate each other, the crystalline lamellae form a more or less continuous super helical structure. It is not clear what material, if any, is present in the central cavity inside the helices, which has a diameter of ~8 nm, and is accessible to the stain in the electron microscopic specimens.

## Implications for biosynthesis

The proposed structure for amylopectin in potato starch raises questions concerning the biosynthesis. Lamellar semi-crystalline structures are also found in synthetic polymers, but the super-helical structure presented here has not been reported before, neither for natural nor for synthetic polymers. The pitch of the helix in starch reported here, originating from the clustering of branch points and characteristic for the botanical source (Oostergetel & van Bruggen, 1989), is

likely to be determined directly by the size and organization of the branching enzyme (possibly in a complex with a soluble starch synthase) involved in the synthesis of amylopectin. It is hard to envisage how the described structure could be formed by a simple apposition of the amylopectin molecules synthesised in the stroma of the amyloplast onto the surface of the granules. So an active role of the starch synthesizing enzymes in the formation of the semi-crystalline network is likely. How the large helices are actually formed remains an open and intriguing question.

## CONCLUSION

This study shows that the crystalline part of amylopectin, one of the two major components of potato starch, forms a regular structural backbone of interconnected helices in the starch granule. About the location of amylose and other components in starch however, very little is known to date. Further detailed structural studies of other starches are necessary to decide whether the presented model is specific for potato starch or more generally valid.

## ACKNOWLEDGEMENTS

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