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The Other Double Helix—The Fascinating Chemistry of Starch

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Organic chemistry texts usually give a description of the chemistry of starch limited to a paragraph or two stating that it is an α-(1→4) linked polymer of glucose and that there are two components present (*1*–*3*). The two components are stated to be amylose and amylopectin, the former being a linear polymer comprising about 20% of the starch by weight, while amylopectin is a branched polymer with frequent α-(1→6) branching points, comprising about 80% of starch. The text may point out that amylopectin is a large molecule with a molecular mass of about 10^9 Da, whereas amylose is much smaller with a molecular mass of about 10^6 Da. The helical structure of starch is sometimes briefly mentioned. This brevity is no doubt due to the need to keep textbooks to a reasonable length, but it leaves out the connection of chemistry to everyday phenomena that makes a subject such as organic chemistry come alive for students.

The chemistry of starch is fascinating for at least three reasons, apart from the fact that starch is the most abundant biomolecule on earth after cellulose and forms a major part of the diet of all human beings. We present this chemistry here in the hope that teachers of organic chemistry will find this article useful as a source for material to make their organic chemistry courses more relevant and interesting, and that some of this information will find its way into future textbooks.

The first point of interest in starch chemistry is the structure of the starch granule itself, which is the form in which starch is laid down by plants as a means of storage. The branching of amylopectin is not an arbitrary feature, but is a necessary aspect of the way in which plants lay down starch in granules in a remarkable partly crystalline form that allows for efficient packing of the starch in the granule. The second point of interest in starch chemistry relates to the molecular-recognition properties of starch itself, the most familiar example being the absorption of iodine from aqueous solution to give the blue color of the test for starch. The third point of interest is the swelling of the starch granule on heating in water above what is known as the gelatinization point, typically about 60 °C. Starch granules can increase in volume by a factor of as much as 100 on gelatinization, and this property of swelling together with the molecular-recognition properties of starch are key features in the cooking of foods involving starches.

The Structure of Starch and Starch Granules

A helical structure for starch was proposed in 1937 (*4*), long before the helical structure for DNA. Crystallographic evidence for such a structure was reported as early as 1943 (*5*). More recent crystallographic studies show that starch occurs in three allomorphs, known as the A, B, and V forms (*6*–*10*). The A and B forms both have left-handed double helices, with six glucose units per turn. The A and B forms appear to differ only in the packing of the starch helices, the A form having a monoclinic unit cell, space group B2 (*6*), and the B form having a hexagonal unit cell, space group $P6_1$ (*7*). The V form is actually a whole family of allomorphs, adopted in the presence of internally absorbed small molecules such as iodine, DMSO, or *n*-butanol (*9*, *10*). All of the V forms have a single-helix structure, also left-handed, with approximately an 8-Å drop per 6 glucose units that constitute one turn of the helix.

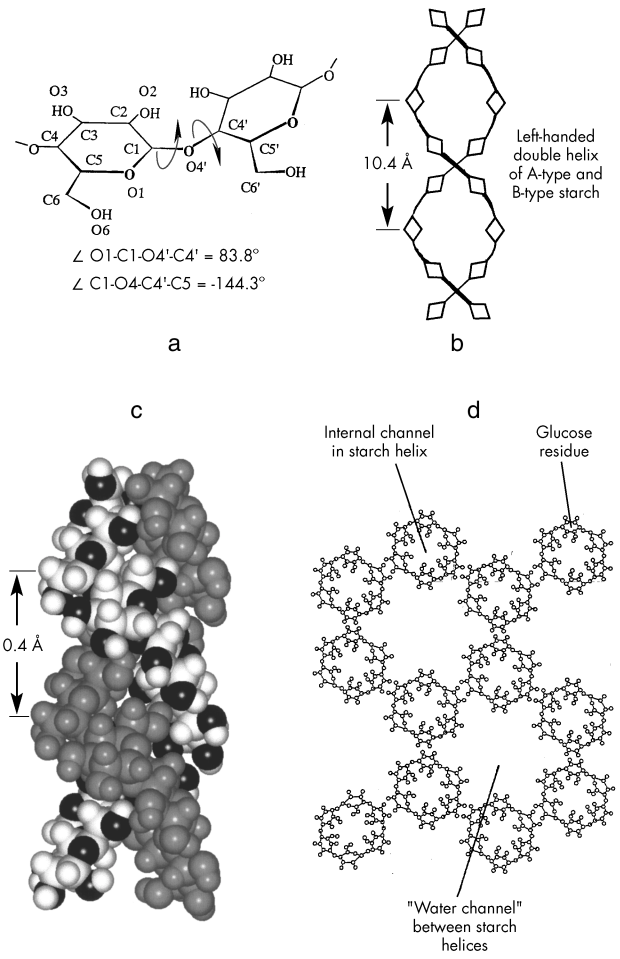


Figure 1. (a) The structure of maltose, showing the torsion angles (curved arrows) across the glycosidic bond holding the two glucose units together (*6*, *7*). If a series of glucose units is connected maintaining these torsion angles across the glycosidic bonds, a left-handed helix is obtained. (b) Two helices entwined give the double helix of A-type or B-type starch shown here. Each diamond represents a glucose unit. (c) A portion of the A-type or B-type starch double helix, which we generated using molecular mechanics modeling. Each strand is 8 glucose units long. One strand has been colored a uniform dark gray to distinguish it from the other. The black atoms in the light strand are oxygen atoms, the large light gray atoms are carbons, and the small near-white atoms are hydrogens. (d) The packing of starch helices in crystalline B-type starch, viewed along the long axes of the helices (*7*) (redrawn after ref *9*). The central cavity of each helix is too small to accommodate water molecules. The large "water channels", each surrounded by six starch helices, are filled to a variable extent with water molecules (not shown).

It is of interest to note that, although we have referred to starch as "the other double helix", starch and DNA are not the only biomolecules with double-helical structures. At least two other polysaccharides, hyaluronic acid and carrageenan, have double helices (*11, 12*). Hyaluronic acid is a mucopolysaccharide that occurs in places such as amniotic fluid and the vitreous humor of the eye (*11*). Carrageenan occurs in red algae (*12*). A triple helix is found in collagen, the protein found in skin, connective tissue, and bones (*13*).

Starch granules show birefringence under polarized light, indicative of long-range internal order. Starch granules give distinctive X-ray diffraction patterns that can be analyzed to elucidate the crystalline structure within. The double-helix structure of type A and B starch is shown in Figure 1. The double helix arises directly from the preferred orientation of two adjacent glucose molecules connected by an α-(1→4) glycosidic linkage (*6, 7*), which is well illustrated by maltose (Fig. 1). Maltose consists of two α-(1→4) connected glucose units, and experimentation with molecular mechanics modeling programs (*6, 7*) shows that there is a strong energy minimum with the O1–C1–O4′–C4′ and C1–O4′–C4′–C5′ torsion angles (Fig. 1) having values of 83.8 and −144.6°, respectively. If one continues to build the model connecting glucose units in an α-(1→4) fashion and maintaining these torsion angles, one obtains one strand of the starch double helix. It is thus remarkable that the structure of the starch helix is implicit in the preferred torsion angles across the glycosidic linkage of adjacent glucose units connected in an α-(1→4) fashion. In the A and B forms of starch the helix has the hydroxymethyl groups of C6 oriented somewhat into the central cavity, and the planes of the glucose units are oriented at a considerable angle to the long axis of the helix. The orientation of the glucose units relative to the axis of the double helix is apparent in Figure 1d. The hydroxyl groups on C6 form hydrogen bonds to the O2 hydroxyls on the other strand of the double helix, which hold the two strands of the double helix together. The orientation of the C6 hydroxymethyl group into the central cavity of the helix means that the central cavity of the A and B forms of starch is too small to accommodate molecules (*7*) (Fig. 2, right). In contrast, the V form has the planes of the rings of the glucose units aligned more nearly in the wall of the helix (*10*) (Fig. 2, left), and has a larger central cavity that is largely hydrophobic. The hydrophobic surfaces of the V-type starch helix arise from the fact that the hydroxyl groups of the glucose units all lie nearly in the plane of the ring (Fig. 2). The regions of the glucose units above and below the plane of the ring thus consist of C–H bonds, which give the starch its hydrophobic surface. It is this hydrophobic central cavity of the V form that gives starch its ability to absorb small linear hydrophobic molecules or hydrophobic parts of molecules. The left part of Figure 2 shows a small linear alkane in the cavity of the V form of starch.

The helical structures are the stable forms of starch in the solid state below the gelatinization temperature, which is normally about 60 °C. Above this temperature in water the helix largely unravels, and the now more-exposed hydrogen-bonding hydroxyl groups of the glucose units form hydrogen bonds to the solvent water, leading to the formation of a gel. On standing, a starch gel will slowly return to a helical folded structure and deposit flocculated particles of starch.

Starch granules have shapes typical of the plants that produce them. The largest granules are found in potato tubers, and may be 100 μm along the long axis. In contrast, starch granules in wheat are smaller, about 30 μm across. The starch granules from potatoes are elliptical, as seen in Figure 3. Micro-

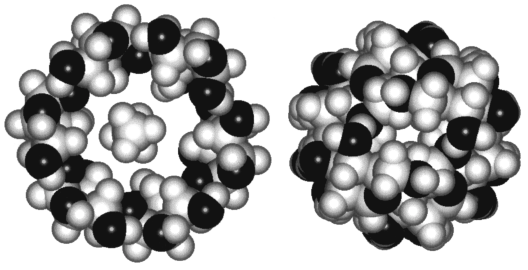


Figure 2. Left: one turn of the type V form of starch, viewed down the axis of the helix. Note how the planes of the rings of the glucose units are more nearly aligned with the axis of the helix, creating a central cavity with an internal diameter of about 4 Å. A short *n*-alkane has been placed in the central cavity to show the good fit, which leads to the ability of starch to absorb small linear molecules such as iodine, *n*-butanol, and fats. The single turn of the starch helix was generated with the aid of a computer modeling program, by joining 6 glucose units using torsion angles about the glycosidic linkages given in ref *9*. The darker large atoms are oxygens, the lighter large atoms are carbons, and the small light atoms are hydrogens. The carbons nearest the observer are the C6 carbons of the glucose ring. The hydroxyls on the C6 carbons hydrogen-bond to those on the C2 carbon atoms of the glucose units in the next turn of the helix. Right: a view down the axis of the helix of type A or type B starch, generated as described for Fig. 1. There is insufficient space in the central cavity of the helix even for molecules as small as water (*7, 8*).

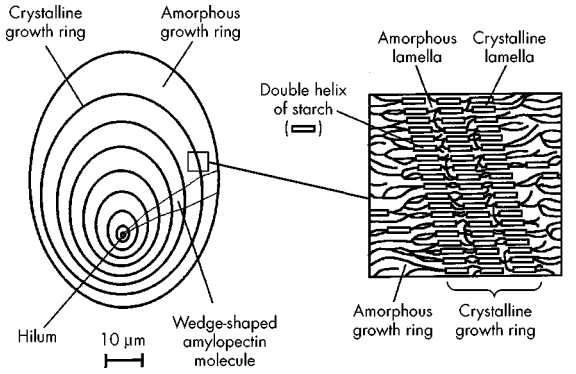


Figure 3. Left: drawing of a potato starch granule, showing the layered structure of alternating crystalline and noncrystalline growth rings. Also shown is the way in which a single amylopectin molecule extends from the hilum to the surface of the granule. Right: enlargement of the layered structure shows the presence of crystalline and noncrystalline growth rings. The crystalline growth rings are subdivided into concentric layers of alternating crystalline and noncrystalline lamellae. The crystalline lamellae contain linear helical sections of amylopectin, whereas the noncrystalline layers contain α-(1→6) branching of the amylopectin. It is the branching in the noncrystalline or amorphous zones that increases the width of the amylopectin molecule and gives it the wedge shape necessary for efficient packing. The amorphous zones of the granule are more susceptible to attack by enzymes such as amylases.

scopically, starch granules have a layered structure, resembling that of an onion. This structure reflects the laying down of alternating layers of crystalline and noncrystalline starch (*8*) (Fig. 3). The point of origin of the starch granule is known as the hilum, and it is from this point that the amylopectin molecules grow. The amylopectin molecules thus extend all the way from the hilum to the surface of the starch granule, accounting for the large size of these molecules.

The packing of molecules growing from a central point to produce a roughly spherical granule will be most efficient if these have an approximate wedge shape in cross section. To achieve this shape the growing amylopectin molecules undergo alternating linear and branching growth to give crystalline and noncrystalline growth rings. This is achieved in the amyloplasts where the granules are formed by alternately using different enzymes that produce either linear or branching growth. Thus at any one time, all the amylopectin molecules in the same starch granule are undergoing *either* branched growth *or* linear growth. It is the layers of branching growth in the amylopectin that periodically increase the width of the molecule and produce the wedge shape. At very high magnification the crystalline growth rings can be seen to have layers (lamellae) within them, which are thought to reflect a daily cycle of growth (*14*).

The role of amylose may be simply that of a packing material, filling in gaps in the structure of the growing granule. It is interesting that in peas, a mutation in an enzyme that lays down starch, known as the *r* mutation, leads to a wrinkled appearance in the pea (hence *r* from Latin *rugosis*, rough) (*15*). The mutation is thought to have occurred in peas around the year 1600. The pattern of occurrence of this mutation in successive generations was one of the inheritable properties of peas that Mendel studied, which allowed him to deduce the laws of genetics. The inefficient enzyme present with the *r* mutation leads to a high amount of amylose and a surplus of unpolymerized glucose in the pea, and a sweeter taste. Peas with the *r* mutation are preferred for the frozen food market because of their superior taste and freezing properties.

The Swelling and Gelatinization of Starch Granules

At room temperature starch normally contains about 20% by weight of water, which occupies the water channel (Fig. 1d). When starch granules are suspended in water and warmed below the gelatinization temperature of about 60 °C, more water reversibly enters the starch structure. One can thus observe a decrease in volume when a suspension of starch is warmed. Water enters the noncrystalline layers of starch more readily than the crystalline layers, and much of the water uptake of starch below the gelatinization temperature probably involves these noncrystalline layers. Above the gelatinization temperature the helix unwinds and becomes solvated forming a gel.

The process of the swelling of starch granules has some puzzling features. One is that the X-ray diffraction pattern indicating crystalline structure within the granule persists even as the granule becomes extensively swollen, and only disappears entirely near the very end of the swelling process (*8*). It seems strange that a crystalline structure could persist while the granule imbibes large quantities of water and swells to as much as 100 times its unswollen size. Another puzzle is the disposition of the starch during the swelling process. The swelling process is very rapid—too rapid to be effectively followed by eye under a microscope. Yet the swollen granule has some mechanical strength (*16*) and appears to have some type of membrane enclosing the starch gel. If the source of heat is removed during the swelling process and the granules are observed microscopically, one sees many granules with their swelling arrested at various stages (R. Hancock and B. Tarbet, unpublished work). Figure 4 shows a sequence of events in the swelling of potato starch granules, reconstructed from granules observed arrested in different stages of swelling. At the onset of swelling, one sees radial cracks originating at the hilum. Coincident with the appearance of these radial cracks, birefringence is lost, and soon thereafter the layered appearance of the granules disappears. As the granule swells further the hilum gives way to a growing cavity, into which extend tendrils that are clumps of amylopectin molecules, or possibly even individual amylopectin molecules. These have been forced apart by the stress generated by the lateral swelling of the outer layers of the granule as hot water diffuses inward. As swelling proceeds the cavity enlarges, and the tendrils shorten and finally disappear as they too become swollen.

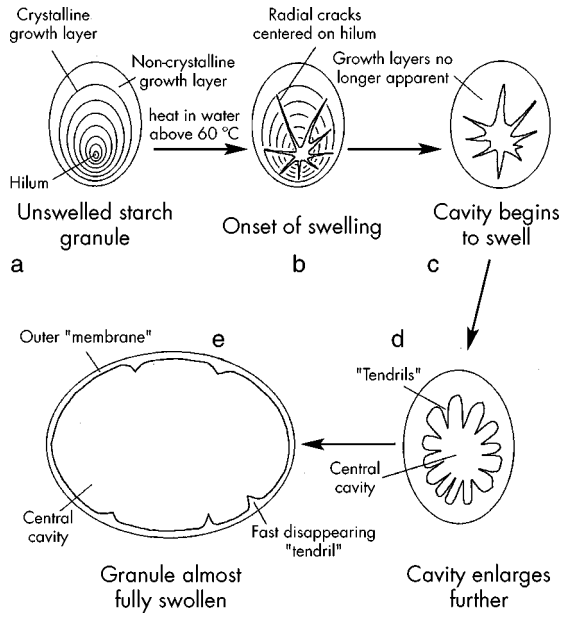
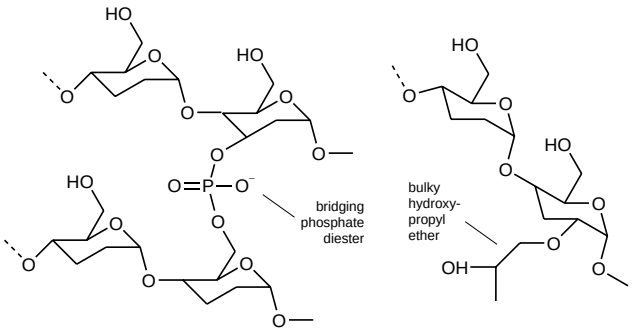


Figure 4. Drawings of the process of swelling of a potato starch granule in hot water. (a) An unswollen starch granule. (b) A granule at the onset of swelling with cracks radiating out from the hilum. The cracks are caused by stress produced by expansion of the outer layers of the granule and follow the lines of packing together of amylopectin molecules. (c) The hilum has given way to an expanding cavity, and the expanding outer layers of the granule have caused further separation of the amylopectin molecules or clumps of molecules along the lines of growth radiating out from the hilum. (d) The process of expansion of the central cavity of the swelling granule has extended further, and the tendrils of the swelling amylopectin molecules are beginning to disappear. (e) The process of swelling is almost complete; only small conical structures on the inner wall indicate surviving tips of the still swelling amylopectin molecules. The Gram–I_2 stain stains the outer "membrane" pink, suggesting that it is predominantly amylopectin, whereas the contents of the inner cavity stain blue, suggesting that this is a gel predominantly of amylose.

It is possible that the persistence of crystalline structure in the swelling starch granules reflects the survival of microdomains of crystallinity within the granules (*14*). Another possibility is that the production of an X-ray diffraction pattern is limited to the nonswollen tendrils of starch extending into the cavity of the swelling starch granule. As discussed below, it is only amylose that absorbs I_2 strongly and gives a deep blue color (*17*). The branching of the amylopectin approximately every 20 glucose units interrupts the linear array of I_2 molecules, which are held together by van der Waals forces. Thus I_2 is much more weakly absorbed by amylopectin than by amylose and gives a much less intense pink color, which is usually masked by the intense blue of the amylose–I_2 complex. With I_2, the outer membrane of the swollen starch granule stains pink and is thus largely amylopectin, whereas the central cavity stains dark blue and is thus an amylose gel (R. Hancock and B. Tarbet, unpublished work). Mechanical agitation of a suspension of swollen starch granules leads to their rupture and the breakup of the outer membrane of amylopectin. Extended agitation leads to breakup of the amylopectin into smaller polymers called dextrins, and a considerable decrease in the viscosity of the gel.

The art of cooking is heavily dependent on the phenomenon of swelling and gelatinization of starch. When bread and cakes and pastries are cooked, the starch is gelatinized. With time, the starch slowly reorganizes itself into a helical structure, at which point the bread is *stale*, and the process is known as *staling* or *retrogradation*. Presumably when the helical structure is reformed, the starch is less prone to attack by amylases in the mouth and so seems tasteless because sugars are not released. The helix also imparts greater rigidity to the starch, leading to the hardness of stale bread. Above 60 °C the gelatinized form of starch is the stable form, so that staling will not occur—although other problems with preserving food at this temperature arise. As the temperature is decreased the rate of staling increases until it reaches a maximum at about 2 °C. Below this temperature the rate decreases again, and bread can be kept fresh in the freezer at about −10 °C. The rate of staling is close to maximum at the temperature of the main part of the refrigerator, so bread will not keep as well there as in the freezer compartment or at room temperature. Enclosing bread in a plastic bag retards the rate of water loss, and so of staling. Making toast with somewhat stale bread will unwind the helix and make the bread taste better, provided there is sufficient water left for gelatinization to occur.

A great deal of ingenuity has been devoted to preventing the re-formation of the helix in cooked starch products, and so of their staling. The prevention of re-coiling of the starch helix is particularly important in the manufacture of frozen foods. On coiling of the starch helix, hydrogen-bonded water is expelled, which leads to "weeping" when the frozen food is thawed. Products for the frozen food market should not undergo retrogradation, which makes them weep as water is expelled when they are defrosted (i.e., they should be "freeze–thaw resistant"). Food chemists have employed the kinds of tactics that would occur to chemists in general to prevent retrogradation of cooked starch. Principally this comes down to steric hindrance to prevent retrogradation in starches modified for food purposes. Two types of strategy have been employed in modified food starches (*18*). One is to cross-link the starch as adipate or phosphate diesters (left-hand structure below). The other is to place bulky groups on the hydroxyl groups of the starch, which can be done with the formation of acetates, hydroxypropyl ethers (right-hand structure below), phosphate monoesters, or octenyl succinate half-esters.



One can also both cross-link *and* place sterically hindering groups on the starch, using both types of strategy. Bread and pastries made with modified starches can remain "fresh" for much longer periods than those prepared with unmodified starches. The modified starches also show increased heat, acid, and shear resistance. Note that the branching in amylopectin produces natural steric hindrance and resistance to staling. That is why "waxy" starches from plants bred to produce starches with > 95% amylopectin show naturally greater resistance to staling.

Complexation of Small Molecules by Starch

The hydrophobic central cavity of the V form of starch allows for the selective absorption ("molecular recognition") of linear molecules such as I_2, *n*-butanol, or fats and phospholipids that have linear hydrophobic alkyl chains (Fig. 2, left). The absorption of I_2 by starch was discussed above.

Controversy has surrounded the structure of starch dissolved in aqueous solution (*18*). One extreme possibility is that the starch has a random coil structure, which means that there are no regions of the polymer that maintain the helical structure. The other extreme possibility is that the starch is all in its helical form. The rate of absorption of I_2 by dissolved starch is diffusion controlled, right up to the point of loading of the starch to its maximum capacity (*19*). Since coiling of a randomly structured polymer of starch is not expected to be so fast, the rapid absorption of I_2 suggests that the starch already has a largely coiled structure in solution. Studies of the viscosity of starch solutions suggest that there are intermittent regions of random structure (*20*). Since the central cavity of the A and B forms appears to be too small to absorb I_2, there is still the problem of how the double helix could convert to a single helix fast enough to absorb I_2.

In cooking, the absorption of fats and phospholipids by starch is of major importance. Cooking with these molecules encourages the formation of starch–phospholipid or starch–fat complexes and greatly affects the texture of the resulting pastry. The formation of these complexes encourages the re-formation of the starch helix and so produces firmer pastries, but the taste sensations under these circumstances do not rely on the release of sugars so much as on the presence of the fats.

Other Uses of Starch

In the U.S. starch is produced predominantly by separation from corn but some is also obtained from other crops, such as potatoes. Starch production involves cold rupture of the cells of the plant material to release the starch granules, which can be washed free of the unwanted material.

Only about 30% of the starch produced in the U.S. and other industrialized countries is used for food purposes. The many uses of starch in the food industry include manufacture of sauces, soups, dressings, ice cream, sweeteners, thickeners, bulking agents, dry mixes, and deep-frozen products. Industrially, starch is used as a sizer (coating and filler) in the manufacture of paper and board, and of textiles. It is also used in the manufacture of adhesives, alcohol, plastics, rubber, pharmaceuticals, and cosmetics, and as a flocculent in water treatment and mining.

Starch science is not in a static state. In addition to the ongoing efforts to produce substituted starches with useful properties, a major current focus is on genetic engineering (*21*). One aim of this research is to learn more about the structure of starch granules by eliminating individual enzymes from the suite of enzymes that control linear and branching growth and the laying down of starch in granules. Further aims are to produce genetically modified starches with useful properties.

Acknowledgment

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