

MECHANISM OF CELL WALL FORMATION

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(WITH THREE FIGURES)

As an outgrowth of x-ray studies on the molecular structure of cellulose walls of plant fibers (**12, 13, 14**), several steps in the process by which the cell wall grows in thickness, seem to have become evident. They are associated with the regularity of molecular arrangement in the wall and with the forces which produce that regularity of structure. As the x-ray data, during the progress of the investigations, made it more and more certain that the structural units are glucose residues, and that they are arranged with the regularity of the molecules in a crystal, the concept became clearer and more convincing that the mechanism of cell wall growth in thickness was associated intimately with the forces which are active in causing that regular arrangement of the residues, that is, with forces similar to those which produce crystallization.

The mechanism which is described in the following pages is concerned with only one phase of cell wall growth; that is, with the increase in thickness of the cell wall. While it may be associated also with the increase in surface area as the cell becomes enlarged and even with the formation of the original wall, those two phases are not included in the present discussion. The problem then becomes restricted to a consideration of the mechanism by which new cell wall material is deposited upon older previously formed cell wall.

Layers in cell wall

Where fiber-shaped cells have been studied microscopically (**1, 2, 11, 15**) the wall has been found to consist of many very thin layers (**1, 2, 11**), which seem to indicate a periodicity in the growth in thickness. Each layer is considered as a deposition of new cell wall material upon the surface of an older layer, and while each thin layer may perhaps have two surfaces available for deposition of new material, we are considering here only the innermost layer of the wall and the surface which is in direct contact with the cytoplasm of the cell; that is, the inner surface of the cell wall. Measurements of these layers show that their thickness is equal to several hundred or perhaps several thousand glucose molecules (**12**). The x-ray studies indicate that each of these thin layers of microscopic visibility is made up of still thinner layers which are invisible and which are only one glucose residue in thickness (**12**). The inner surface of the cell wall would be, then, one side of such a layer of glucose residues.

Relation of glucose to cellulose

That the newly deposited material is composed of glucose residues is inferred from the works of various investigators. The new layers have been shown repeatedly through microtechnical work to be composed of cellulose, and cellulose has been shown, through the work of chemists (7, 4) to be composed of glucose residues. The residue is a $C_6H_{10}O_5$ type of carbohydrate, an anhydrous form of glucose; but concerning the nature of the material from which the residue is deposited, that is, the material immediately preceding its appearance as cellulose, there is very little discussion in the literature. No experimental evidence is available to show that the anhydrous glucose units of the cell wall are derived directly from glucose molecules. The assumption of its glucose origin is made from the reverse of that reaction; that is, since cellulose may be converted into glucose, the assumption is that glucose in the cell is converted into cellulose. The former is a common laboratory hydrolysis experiment; the latter, glucose to cellulose, a condensation reaction in which water is split off, has never been accomplished *in vitro*. It is not known then whether the glucose molecule, originating through photosynthesis, becomes transformed first to some component part of the protoplasm and then re-transformed into the carbohydrate, cellulose, or whether it is transformed directly into cellulose.

In developing the mechanism of cell wall growth, we are accepting two conclusions which seem to be reasonable: first, that the deposition of new material is made at the interface between the cytoplasm and the wall; and secondly, that glucose is transformed directly into cellulose. While the mechanism is more readily described by accepting these two concepts they might vary considerably without invalidating the mechanism proposed.

Since the process involves a transformation of glucose molecules at the surface of the cell wall into cellulose molecules which become component parts of the wall structure, a detailed picture of the molecular situation at the interface is necessary.

Molecular structure of cell wall

Interpretation of x-ray data shows that the cell wall of plant fibers is constructed of units or building blocks of molecular size which have an orderly arrangement in three dimensions (6, 10, 5, 12). The unit of structure proves to be an anhydrous residue of β d-glucose (13, 9, 7). The empirical formula for the glucose molecule is $C_6H_{12}O_6$; for the anhydrous residue, $C_6H_{10}O_5$. Spatially these molecules or groups of atoms are three dimensional structures in which the atoms have definite positions allocated to them (13, 9). The distances between the atoms, and their location with reference to one another, are known with sufficient definiteness from x-ray

data (3) and from chemical experimental data (4) to allow a fairly accurate model of the molecule to be constructed. Fig. 2 is a projection of such a model of a β d-glucose molecule (13, 9). The carbon atoms are numbered 1 to 6, and the oxygen atoms are indicated by solid black circles. The positions of only two of the 12 hydrogen atoms are shown, as a matter of convenience. These two are represented by the smaller circles attached to the oxygens, forming the OH groups which in turn are attached to the carbons numbered 1 and 4. This glucose molecule becomes an anhydrous residue when the OH is removed from C_4 , and the H from C_1 .

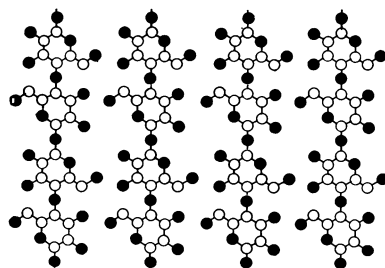


Fig. 1. View of inner surface of cell wall showing four chain molecules. Only four glucose residues of each chain are shown. Solid black circles represent oxygen atoms; light circles, carbon atoms; hydrogen atoms not shown.

In the wall of the fiber each anhydrous residue has a very definite position with reference to the neighboring residues such that the spacing between them in a given direction is uniform. That may be brought out more clearly by referring to fig. 1, where sixteen residues are shown, drawn practically to scale. The distances from center to center of the residues are proportional to the spacings determined from x-ray work with fibers, and their relative positions also are in accord with the x-ray data. They represent, however, a three dimensional structure, and a more truthful picture is obtained when we think of the odd numbered carbons as being raised above the page and the even numbered as being in the plane of the paper. Alternate oxygens are to be considered also as raised above the plane of the paper.

The picture of the structure as given in fig. 1 represents a surface view of the cell wall, such as a minute observer might see, whose senses were attuned to atomic dimensions, if he were inside the fiber looking directly at the inner face of the cell wall. The units or residues would appear to be attached to one another, lengthwise of the fiber, through an oxygen atom acting as a bridge between each two residues. The effect would be that of long chains with the residues acting as links of the chain. The chains themselves, however, because of the greater distance which separates them from one another, would seem to be suspended in space. Fig. 1, then, would

represent a section of the inner surface of the wall showing only parts of four chains, each part containing only four residues.

Inner surface of cell wall

On looking through that surface layer of chains, other similar layers would be seen, all of them parallel to the surface and separated from one another by uniform distances. The distances separating the layers would be nearly the same as those separating the chains. We are especially interested in the layer which forms the inner surface of the cell wall, because it is upon that layer that the new material is deposited. New residues which may be laid down on this surface become arranged in the same orderly manner as the previously deposited residues; that is, they take up positions which result in an extension of the lattice structure towards the interior of the cell.

Inner surface similar to crystal face

The regularity in arrangement of the structural units of the cell wall as revealed by x-ray studies is essentially the regularity of crystal structure; that is, the residues in the cell wall are directly comparable to the molecules in a crystal as, for example, the molecules of sugar in a crystal of sugar. The residues, like the sugar molecules, are not only spaced in an orderly manner but are also oriented in a definite way with respect to one another. It would seem then that forces are involved in locating and orienting the residues in the cell wall, which are comparable to those acting in a similar way on the sugar molecules during the formation of a sugar crystal.

The general conception concerning these forces is that they are due to the electrical conditions occurring in the component atoms, which set up electrostatic force fields around the molecule; and that these force fields are responsible for the cohesion between the molecules in the crystal (8). In some way these force fields produce the orientation of the molecules when they are being deposited on the crystal face; and they also determine the location of the molecule. The position which the molecule is forced to take is one of minimum potential energy which is also the position of a unit of the crystal lattice.

Since the glucose residues act as lattice units of the cell wall, one is led to think that the force fields which determine their position and orientation are similar to those acting when molecules are deposited on a crystal face. The inner surface of the cell wall then may be considered as similar to a crystal face as regards the distribution of forces which are involved in the deposition of new lattice units.

Glucose molecule and its anhydrous residue

There is, however, this difference, that the sugar molecule has very probably the same structure in solution as it has after it is deposited as a unit of the sugar crystal; while the glucose molecule in the cytoplasm is slightly different in its structure from the residue which acts as the unit in the cell wall. As a matter of fact there is a great probability that the β d-glucose molecule is only very slightly different from its anhydrous residue, the difference between them being the loss of one OH group and one H atom from carbons number 1 and 4, respectively, or vice versa. That may be visualized readily from fig. 2. It may be that the possibility of the formation of cellulose is due to that particular difference and similarity. There seems to be no fundamental reason for thinking that the component atoms of the molecule and of the residue differ in their general spatial relations to one another, although that has not been definitely proved.

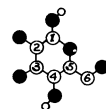


FIG. 2. A single glucose molecule. The carbon atoms are designated by number. Only two hydrogen atoms are shown.

Transformation of glucose to cellulose

When we accept the general similarity of the two, and assume that the glucose molecule could be deposited on the surface of the cell wall as though it were a residue, a very interesting state of affairs is brought out. The molecule would probably take a position of minimum potential energy, which would be the position of a unit of the lattice; also it would be oriented nearly, if not exactly, as a residue would be if in that place. That may become clearer by imagining a glucose molecule (fig. 2) as finding the proper position over a surface such as represented by fig. 1, and thinking of the molecule as the starting point of a new layer. A second molecule may be expected to fit into an adjacent position. This could be represented by substituting fig. 3 for fig. 2. The force fields, or we may say the cohesion, should hold these two molecules practically as firmly to the surface layer as would be the case if they were anhydrous residues. When the molecules of fig. 3 are forced into the proper orientation by forces of the surface layer, the two OH's attached to the numbers

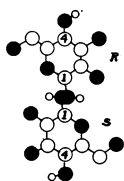


FIG. 3. Two glucose molecules placed as they are oriented in the cellulose chain, to show the position of the OH's at time of condensation. Smaller circles represent hydrogen atoms.

1 carbons are brought into close proximity. That is decidedly an advantageous spatial relation for a condensation reaction to take place. The result of such a reaction is the formation of a water molecule, $\text{OH} + \text{H} = \text{H}_2\text{O}$; and at the same time, the formation of an oxygen bridge between the two glucose

residues in which the remaining O is used. The paragraph may be summed up by saying that the force fields, resulting from the regular distribution of the glucose residues in the wall, have made the conditions favorable for a condensation reaction, such as the organic chemist usually expects when two OH groups are brought close together.

Relation of lattice to long chain formation

The conditions are made favorable, however, for the reaction to take place in one direction only. It may be seen in fig. 1 that the distance from center to center of the residues is too great laterally for condensation to occur between adjacent units of two chains; while in a lengthwise direction of the chains the distance is extremely favorable for the reaction. That would allow an indefinite number of glucose molecules to be brought into position in which the condensations would be made probable; and the formation of a long straight chain of residues linked by oxygen bridges might readily follow.

Relation of glucose structure to chain molecule

The structure of the β d-glucose molecule also favors the straight chain formation. Evidence from both chemical (4) and x-ray work (13, 9) shows that the oxygen bridges are associated with carbons number 1 and 4. These two carbon atoms are found to be located on opposite sides of the glucose molecule model. That location places them in positions in the molecule which are decidedly favorable for the condensation reaction, and in positions which permit of a straight chain formation.

Evidence of a third factor

Although the molecular and atomic arrangements and dimensions all tend towards the formation of cellulose chain molecules, the mechanism described above cannot account for all of the observed facts. The mechanism mentioned requires only two factors, (1) the cellulose surface and (2) the presence of glucose molecules. It is well known, however, that glucose may be present in a cell and no growth take place in the thickness of the wall. A third factor, then, must be involved in the growth. It is also well known that no growth in thickness of the cell wall occurs after the protoplasm has become inactive, although glucose may be present. The third factor, then, must be associated with some reaction which can occur only when living protoplasm is present. Another well known fact, that the wall may grow on one side of the cell and not on another side, indicate that the third factor is localized in the cell. And since chunks of cellulose do not occur at random throughout the protoplasmic mass inside the cell it would seem that the third factor is localized only at the interface between the cytoplasm and the wall.

Suggestions as to the nature of that third factor are rather meager. Speculation suggests many lines of approach. Careful studies of the conditions inside of the cell at the time the cell wall is being thickened, studies with a view point to determining the concentration of glucoses, and the forms in which they occur, the rate of respiration, the water content of the cytoplasm, the presence of dehydrating bodies, etc., may throw some light upon the remaining parts of the mechanism.

The use of the expression "third factor" or "third body" is, of course, merely symbolical of whatever process or processes must be carried on to complete the condensation reaction which was made possible by the surface forces of the inner face of the cell wall.

Summary

When considering the growth of a cell wall, three stages in the process may in general be recognized: (1) the formation of the original or first layers of cellulose; (2) the surface area growth of the wall as the cell becomes larger; and (3) the increase in thickness of the wall. The third stage only is under consideration in this paper. An attempt has been made here to show that inherent in the molecular structure of the wall are forces which must share very largely in the process of building up the wall itself in thickness at least.

An outstanding feature in the mechanism of thickness growth, it may be pointed out, is the part played by a *surface of regularly spaced molecular units*. The necessity of a surface of some kind on which cell wall material may be deposited is in accord with microscopic studies; but concerning the molecular structure of the surface, only one kind of foundation surface, that of the cellulose wall itself, has so far been investigated. The preceding discussions, then, refer only to cellulose surfaces in which the regularity of arrangement of the structural units, β d-glucose residues, suggests a comparison with crystal surfaces in which large molecules act as structural units. From that comparison one is led to conclude that the forces of crystallization form a part of the mechanism by which glucose molecules become converted into cellulose. Upon following that line of thought farther, it was found that on account of the particular structure of the glucose molecule an opportunity was provided for a condensation reaction which would produce long straight chains of residues, or cellulose molecules.

It was pointed out that crystallization forces alone could not account completely for the transformation of glucose to cellulose; that a third factor which seemed to be associated with living protoplasm was necessary. No discussion of the nature of that third factor is given.

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