Korean J. Appl. Microbiol. Biceng.
Vol. 6 No. 4, 197 (1978).

▶ Review ◀

Properties of Cellulose as an Enzyme Substrate

효소기질로서의 섬유소의 성질

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(Received Sept. 20. 1978)

ABSTACT

Voluminous studies have been carried out on the utilization of cellulosic materials as a raw material for the production of sugar or single cell protein. The structural complexity of the substrate generated problems in the research works as well as in the application of the results obtained in the researches.

This review outlines the physical and chemical properties of cellulosic materials obtained from researches in the field other them biology.

Cellulose is the main constituent of the plant cell wall. Like all the natural organic compounds, cellulose returns to atmospheric carbon dioxide by oxidation. Though a small part of 2.2 ×1011 tons of cellulose produced annually is utilized by man, vast amount of it is decomposed by microorganisms in nature. These organisms utilize the polysaccharide as their energy and carbon sources, after hydrolizing it into simple sugars such as glucose and cellobiose. The biological degradation of cellulose depends on the accessibiliy of the reactive sites to the active center of the enzymes. Cellulose is water-insoluble and has a crystalline form, thus the reactive sites, glucosidic bonds, available to the enzyme are very restricted. To make it worse cellulose co-exists with lignin which is very resistant to the biological degradation. These unique characteristics of the substrate make it very complicate to study the reaction mechanism of the bilological cellulolysis as well as to utilize cellulosic materials as microbial substrate.

The properties of cellulosic materials will be discussed in relation to their susceptibilities to biololgical degradation.

Composition of Cellulosic Materials

The chemical constituents of plant cell wall include cellulose, hemicellulose, lignin, a wide variety of extraneous materials and inorganic materials(Table 1). Lignin and hemicellulose are the major non-cellulosic compounds in cellulosic materials. High concentrations of ash are found in some plants. This is mainly siliceous in rise straw. In addition to the complexity of cellulose itself the contents of these materials determine the digestibility of cellulose in the plant cell wall, since they form a matrix surrounding the cellulose microfibrils.

Hemicellulose is relatively short hetero-polysaccharides which consist of pentoses (xylose, arabinose etc.), hexoses (glucose, mannose, galactose etc.) and uronic acids of glucose and galactose. Generally hemicellulose is in non-crystalline form and is easily hydrolyzable by enzymic actions,

Lignin is a complex three-dimensional polymer

Table 1. Compositions of Plant Materials. (%)

Constituent	Cotton	Birch	Spruce	Bagasse	Rice straw
Helocellulose	94.0	77.6	70.7	70.5	56.2
Cellulose	89.0	44.9	46.1	46.0	
Hemicellulose	5.0	32.7	24.6	24.5	
Lignin	0.0	19.3	26.3	19.9	12.4
Crude protein	1.3	0.5	0.2	19.9	3.6
Extractable extraneous material	2.5	2.3	2.5	3.5	6.5
Ash	1.2	0.3	0.3	2.4	13.7

formed by an alcohol groups attached to benzene rigs and polyphenols. The exact configuration of lignin is not known though the monomers of the polymeric compound are known[1]. As mentioned already lignin is one of the most resistant materials in nature to the biological degradation. Some microorganisms such as white-rot fungi are reported to attact lignin, but at extremely slow rates. It took more than several weeks to obtain a measurable degradation of lignin used in the culture medium for the lignolytic organism[2]. Lack of knowledge on the structure of lignin and the slow rates involved make it difficult to study the mechanism of enzymic hydrolysis of lignin. Strong alkaline solutions solubilise lignin by saponification.

Ash is a minor element in the majority of cellulosic materials, however, in some cases the ash content is greater than 20%[3]. The mineral components sometimes stimulate the growth of microorganims on the cellulosic materials containing them. but high concentration is responsible for poor digestibility by enzymes.

Structure of Native Cellulose and Polymorphs of Cellulose

Cellulose is a polymer of 1-4 linked β-D-glucose residue ranging between 30 and 15,000 units of glucose in size. Glucose molecules in cellulose are stearic ally in the chair form of pyranose. X-ray analysis showed that native cellulose has a highly crystalline form[4]. Infra-red spectroscopy shows that there are few free hydroxyl groups in native cellulose. It appears that most of the hydroxyl groups are engaged in hydrogen-bondings[5]. Hydrogen-bondings are observed between as well as withincellulose molecules[6]. (Fig. 1).

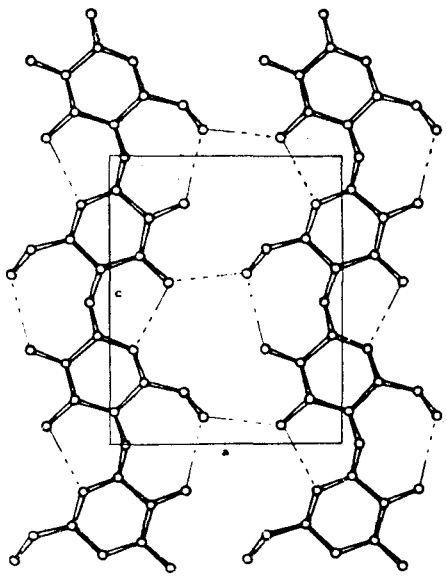

Fig. 1. Unit Cell Structure of Cellulose Showing Intra and Inter-molecule Hydrogen-bonding.

Hydrogen-bonded cellulose molecules from elementary fibrils which are about 35 Å in diameter (Fig. 2b). The elementary fibril which contains about 40 cellulose molecules was regarded as a uniform structure in native cellulose of different origins[7]. However, high resolution electron microscopy had revealed that there are smaller fibrils (subelementary fibrils) whose diameter is from 15 Å to 20 Å[8]. Methylation studied of the free hydroxyl groups of cellulose showed that the elementary fibrils are the basic crystal structure of native cellulose and have an average cross section 8×10 cellulose chains. This cross section measures

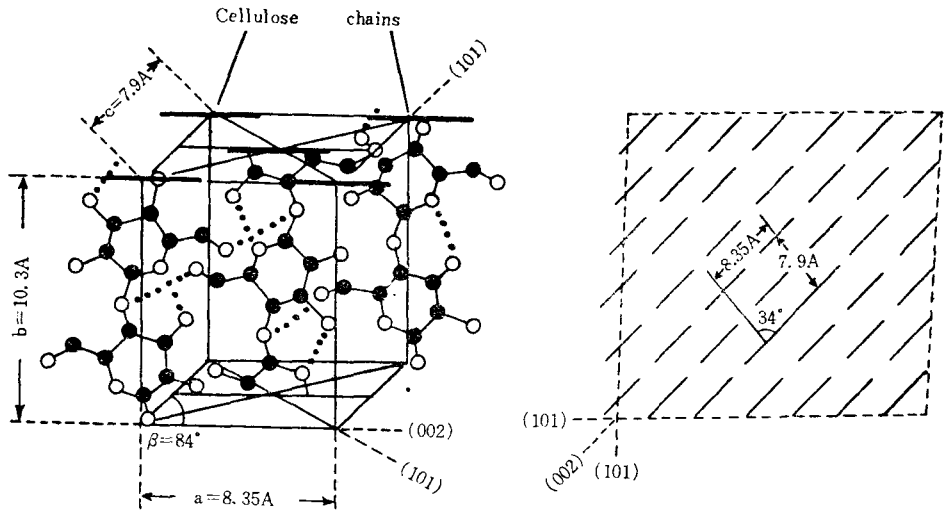

Fig. 2. (a) Unit Cell of Cellulose I.
(b) Cross Section of an Elementary Fibril Showing the Arrangement of the Unit Cell.

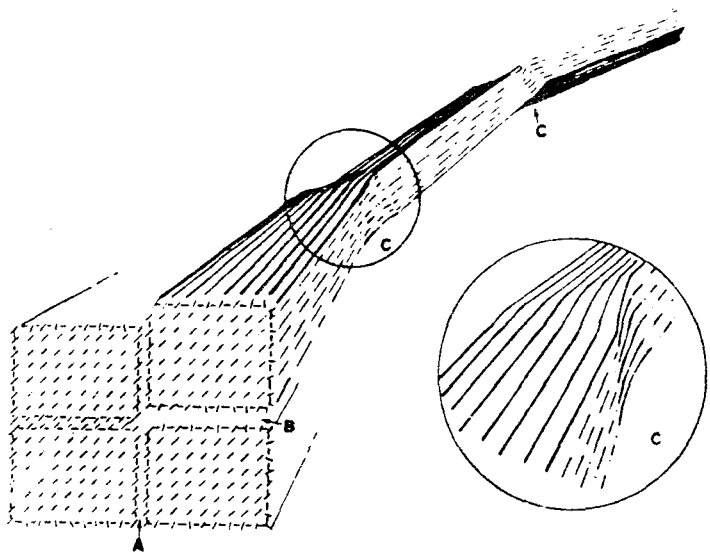

Fig. 3. Schematic Representation of the Elementary Fibril to Illustrate the Crystalline Elementary Fibril Theory of the Microstructure of Cellulose and to Show (A) Coalesced Surfaces of High Order, (B) Readily Accessible Slightly Disordered Surfaces, and (C) Readily accessible Surfaces of Strain-distorted Tilt and Twist Regions.

approximately 40×50 Å[9]. Within the elementary fibril, crystalline and amorphous regions are repeated. The amorphous regions are responsible for the flexibility of cellulose fibre. The elementary fibrils in turn form microfibril whose dimension is 50×100 Å in its cross section.[10] (Fig. 3)

X-ray diffraction studies showed cellulose to be a periodic structure of 10.3×8.2×7.9 Å. This spacing indicates two cellobiose molecules which is termed as a unit cell (Fig. 2a). Fig. 2-b shows the position of a unit cell in the elementary fibril.

The unit cell structures of native celluloses from different sources have been considered to be exactly

the same. Some reports, however, have suggested that the bacterial (*Acetobacter xylinum*) and algae (*Valonia Ventricosa*) cellulose have different unit cell dimensions from other celluloses. This super unit cell has a and c axes which are twice as song as normal[11,12].

Dry cellulose can exist in several crystallographic forms. Their differences are due to the changes in the relative positions of glucose molecules within the unit cell. The native cellulose is in I from, and cellulose II, III and IV form are generated forms of cellulose[13]. The dimensions of the chief polymorphs of cellulose are shown in Table 2.

Table. 2. Parameters for the Basic Unit Cells of the Chief Polymorphic Forms of Cellulose[14].

Polymorphic form	Axis(Å) a	b	c	β (degree)	Density (g/cm^3)
I	8.2	10.3	7.9	84	1.62
II	8.2	10.3	9.2	63	1.62
III	7.7	10.3	9.9	90	1.61
IV	8.1	10.3	8.0	90	1.61

Structural Changes in Cellulose by Chemical and Physical Treatments

The structure of cellulose can be changed by chemical and physical treatments. Without this property cellulose cannot be used as the raw materials for paper and textries as it is. For example cellophane is manufactured by recrystallization after pulp is dissolved in a suitable solvent. Many other products can be named such as rayon, vegetable perchemented paper etc.

Table 3. Swelling Agents of Cellulose[15].

Alkali hydroxide
LiOH, NaOH, KOH, RbOH etc.
Salts in strongly alkaline solution
Sodium zincate, Potassium zincate, Iron-sodium tartarate etc.
Inorganic Acids
H_3PO_4, H_2SO_4, HCL $HNOO_4$, HNO_3 etc.
Inorganic salts
LiCI $CaCI_2$, $ZnCI_2$, LiBr, $CaBr_2$ etc.
Amines and related compounds
ethylene diamine diamine etc.
Mixed reagents
Metal-amine complexes(Cu, Zn or Ni in amine solution)

The native cellulose I as well as other form of cellulose transforms into amorphous cellulose by treating with various swelling agents or dissolving in inorganic and organic solvents. They are listed in Table 3. [15].

As shown in Table 4 the unit cells of the swollen cellulose have much bigger dimensions in axes a and[15]c. The swelling agents are believed to interupt the hydrogen-bondings in the cellulose, and form cellulose complex.

The swollen amorphous cellulose is recrystallized by drying it. In the recrystallization process the

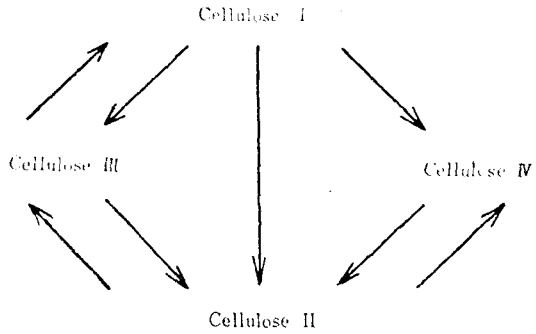

Fig. 4. Established Interconversion Possibilities between the Chief Polymorphs of Cellulose[17].

humidity and the temperature are the factors determining the polymorphic form of the recrystallized cellulose[16]. Fig. 4 shows the interconversion possiblity between the chief polymorphs of cellulose [17].

Table 4. Unit Cell Dimensions of Swollen Cellulose[15].

Cellulose	Axis(Å) a	b	c	β (degree)
I	8.2	10.3	7.9	84
II	8.2	10.3	9.2	63
Na-I	25.6	10.3	13.2	40
Li-I	9.0	10.3	17.5	30
HNO_3-1	12.2	10.3	9,7	53
$HCLO_4$-I	16.5	10.3	10.7	93
Ethylene diamine-I	12.2	10.3	12.3	43

Pore Size in Cellulosic Materials and Molecular Size Cellulolytic Enzymes

Provided an enzyme disrupts the cellulose crystal structure prior to the hydrolytic reaction, it can be said that the accessibility of the enzyme to the crystals is prerequlisite for the whole reaction.

The size of void capillary between the crystals in cellulosic materials has been measured in an experiment using water soluble high polymer. The pore size in cotton was measured to be 10~20 Å [18]. Pores in black spruce wood were reported to be slightly larger than in cotton, and increased according to the degree of pulping, which led to the removal of non-cellulosic materials. The median pore width of kraft pulp is 50~60 Å when the yield of pulp is 40% [19].

Based on reported molecular weight, the size of celluloytic enzymes were calculated either as sphere of 24-27 Å or as elipsoids of 12×79~42×525 Å [10].

Because cellulolytic enzyme molecules are bigger than void capillaries in cellulosic materials (Fig 5), elementary fibrils, the basic crystal structure of cellulose are not readily available to the enzymes[20].

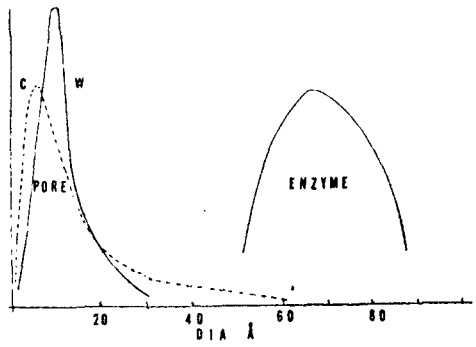

Fig. 5. Comparison of Pore Diameter of Cell Wall Capillaries of Cotton (C) and Spruce wood (W), With Diameter of Cellulase molecules[20].

Physical and Chemical Constraints on the Susceptibility

From the discussion on the properties of cellulosic materials, we have some idea on the difficulties in the biological hydrolysis of cellulose. Factors affecting the susceptibility of cellulose to enzymic hydrolysis will be discussed briefly.

1. Accessibility of Substrate Surface to Enzymes

The accessible surface of cellulose is defined by the size, shape and surface properties of the substrate. In other words the substrate with larger and more capillaries are more susceptible than those with smaller and less capillaries.

2. Degree of Crystallinity

It is well known that amorphous cellulose is readily hydrolysable while the crystaline one is more resistant to enzymes. A significant increase in crystallinity was observed during hydrolysis by enzyme as by acids. As the less crystallised fraction of the subatrate is hydrolyzed, the residual material becomes more crystalline[21].

3. Polymorphs of Cellulose

The cellulose I is more resistant to the enzyme

than regenerated cellulose II, III and IV. Since changes in crystallinity are inevitable during swelling and recrystallization, it is difficult to determine whether their altered susceptibility to enzyme is due to changes in crystallinity or due to changes in the unit cell dimension. It was argued that cellulose II is more resistant to enzymic hydrolysis than cellulose I, because the former is denser than the latter.

4. Non-cellulosic Components

The effects of lignin and hemicellulose on cellulolysis were already discussed. In addition to them plant residues contain a wide variety of non-cellulosic materials mostly extractable by neutal solvent such as benzene, acetone, alcohol and water. They can be grouped into ① various substances deposited in the fine capillary structure ② specific enzyme inhibitor. The enzyme inhibitors are found mostly in fresh plant materials and are more effective on the enzyme which reduces the crystallinity of cellulose[22].

Pretreatment to Increase Digestibilty

Various methods have been attempted to increase the digestibility of cellulosic materials. The effects of these treatments on the cellulosic materials include reduction in crystallinity of cellulose, removal of lignin and ash, and/or both.

1. Chemical Treatment

Sodium hydroxide has been most commonly employed for this purpose[23~29]. As mentioned earlier strong alkaline solutions are known to remove lignin by saponification[24] as well as to swell cellulose[30,31]. Some attempts have been made to use weaker and cheaper alkaline solutions such as ammonium hydroxide, but the resutls were not satisfactory[32,33].

Some bleaching chemicals have been examined as possible agents to improve the digestibility of lignocellulose such as hypochlorite[29] and peracetic acid[34].

Mineral acids have been used for the same purpose with less convincing results.[28] Unlike alkali acids cannot remove lignin, though they do reduce crystallinity of cellulose.

By alkali treatment the hemicellulose fraction is lost since alkali solution dissolves hemicellulose as well as lignin. There is an alternative approach known as Canadian process to save the hemicellulose fraction[26].

After treatment in aqueous solution, sample drying decreases the digestibility by enzyme[34,36], X-ray crystallography showed the increase in crystallinity by drying[37].

2. Physical Treatment

Ball milling destroys the lignocellulose structure making it highly susceptible to enzymic attack.[35] It was also reported that lignocellulose ground using a vibrating millat high temperature (220°C for 25 min.) is highly susceptible to cellulolytic agents[38-40].

Irradiation by X-ray[41] and steaming[25] were both reported to be effective agents in increasing susceptibility to enzymic attack.

요 약

섬유소 또는 이를 함유하는 물질을 원료로 당 혹은 단세포단백질을 생산하고자 하는 수 많은 연구가 시도되었다. 기질인 섬유소 혹은 이를 함유하는 물질이 구조가 복잡하여 수용성인 일반 효소기질과 달라서 이를 이용하고자하는 연구에 많은 문제점을 갖고 있으며 실험실 연구 결과를 실제 응용하는 면에서도 어려움을 주고 있다.

본고에서는 이를 물질의 생물학적 분해에 관계되는 물리 및 화학적인 성질을 간단히 살펴보았다.

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