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THE BIOSYNTHESIS OF CELLULOSE

R. MALCOLM BROWN JR.

Department of Botany
The University of Texas at Austin
Austin, Texas 78713-7640, USA

ABSTRACT

Cellulose is one of the major commercial products of Sweden and constitutes the most abundant of the natural polymer systems. Thus, it is of interest to review the molecular design and architecture of cellulose with particular reference to the controls of its biosynthesis. The bioassembly process is highly ordered and structured, reflecting the intricate series of events which must occur to generate a thermodynamically metastable crystalline submicroscopic, ribbonlike structure. The plant cell wall is an extremely complex composite of many different polymers. Cellulose is the "reinforcing rod" component of the wall. True architectural design demands a polymer which can withstand great flexing and torsional strain. Using comparative Hydrophobic Cluster Analysis of a bacterial cellulose synthase and other glycosyl transferases, the multidomain architecture of glycosyl transferases has been analyzed. All polymerization reactions which are processive require at least three catalytic sites located on two different domains. In contrast, retaining reactions with glycosyl transferases require only a single domain and two sites. Cellulose synthase appears to have evolved a mechanism to simultaneously bind at least three UDP-glucoses and to polymerize, by double addition, two UDP-glucoses in such a manner that the 2-fold screw axis of the β-1,4-glucan chain is maintained. Thus, no primer is required as the glucose monomers are added two-by-two to the growing chain. At the next higher level of assembly, the catalytic sites simultaneously polymerize parallel glucan chain polymers in close proximity so that they will favorably associate to crystallize into the metastable cellulose I allomorph. Recent energy analysis suggests that the first stage of this associ-

ation is the formation of a minisheet through van der Waals forces, followed by layering of these minisheets to form the crystalline microfibril. In native cellulose biogenesis, the microfibril shape and size appear to be determined by a multimeric enzyme complex (TC) which resides in the plasma membrane. This complex, known as a terminal complex, was discovered through electron microscopy of freeze fracture replicas. The entire complex moves in the plane of the fluid plasma membrane as the result of polymerization/crystallization reactions. The assembly stages for native cellulose I are coordinated on a spatial/temporal scale, and they are under the genetic control of the organism. This might lead one to conclude that cellulose I could only be assembled with Nature's indigenous machinery; however, this is not the case. Recently, in collaboration with Professor Kobayashi and his colleagues in Sendai and Tokyo, we have synthesized cellulose I abiotically under conditions very different from those in the living cell or from isolated cell components. Purification of an endoglucanase from *Trichoderma* which serves as the catalyst and the addition of β-cellobiosyl fluoride as the substrate in acetonitrile/acetate buffer has led to the assembly of synthetic cellulose I. Although natural and synthetic assembly pathways are very different, there are similar, underlying fundamental mechanisms common to both. These mechanisms will be discussed in relation to the more thermodynamically stable allomorph of cellulose (cellulose II) first demonstrated by Professor Rånby in 1952. The evolution of cellulose biosynthesis will be summarized in terms of the demands for maintaining optimal cellular environments to generate the complex macromolecular assemblies for cell wall biogenesis. Nature provides an exceptional model for cellulose biosynthesis that will lead us toward the biotechnological production of improved natural cellulose as well as synthetic cellulose and its derivatives.

INTRODUCTION

We have gathered in Stockholm to honor Professor Bengt Rånby, a colleague whose long and productive career has traversed the domain of polymer science. Professor Rånby's significant contributions in the field of polymer chemistry and more specifically his work on the structure of cellulose, support the idea that a topic covering the biosynthesis of cellulose is appropriate for the theme of this symposium, "Nature as a Model of Molecular Design of the Polymeric Materials of Tomorrow." In terms of the massive quantity of natural polymer biosynthesis, we know that *Nature is alive and well* with respect to cellulose. This biopolymer is the most abundant macromolecule on earth and is synthesized by plants, fungi, algae, bacteria, and several animals (Table 1).

Although cellulose is polymerized from a simple sugar, glucose, its biosynthesis is complex and only incompletely understood. In this presentation the molecular architecture of cellulose will be introduced, followed by an update on the details of its natural biosynthesis. A comparison of native and synthetic cellulose production will be made, followed by a discussion of evolutionary implications and recent

TABLE 1. Cellulose Found among Living Organisms. Several Representative Genera Which Have Been Studied for Cellulose Biosynthesis are Included

Prokaryotic organisms:
- Gram-positive anaerobic bacteria *Sarcina*
- Purple bacteria *Acetobacter*, *Rhizobium*, *Alcaligenes*, *Agrobacterium*

Eukaryotic organisms:

A. Photosynthetic organisms [66]
- Chlorophyta (green algae)—*Oocystis apiculata*, *Valonia*, *Boergesenia*, *Chara*, *Mougeotia*, *Coleochaete*
- Charophyta (stoneworts)—*Chara*, *Nitella*
- Phaeophyta (brown algae)—*Pelvetia*
- Chrysophyta (yellow-green, golden-brown algae, and diatoms)—*Vaucheria*, *Pleurochrysis*
- Rhodophyta (red algae)—*Erythrocladia*
- Vascular plants:
  - Mosses, liverworts, ferns, angiosperms, gymnosperms, etc.—*Funaria*, *Arabidopsis*, *Zea mays*, *Gossypium*, *Pinus*, *Phaseolus*

B. Nonphotosynthetic organisms:
- Protists—*Dictyostelium discoideum*
- Fungi—*Saprolegnia*, *Allomyces*, *Achlya*
- Animals:
  - Tunicates—*Metandrocarpa*, *Hyalocynthia* [67]
  - Humans—(associated with the disease scleroderma) [68]

molecular genetics research, ending with perspectives on future research directions in the field of cellulose research.

THE MOLECULAR ARCHITECTURE OF CELLULOSE

What is "cellulose?" In the past, confusion and misunderstanding have prevailed when the definition of "cellulose" was considered. In 1839, Anselme Payen, a French industrialist and chemist [1], coined the term "cellulose" to describe an acid-resistant substance obtained by treating wood with nitric acid. Payen found that irrespective of its origin, cellulose had the same chemical composition ($C_6H_{10}O_5$). The modern definition of cellulose must take into account not only the *composition* of the polymer but also its *linkage, molecular weight, and crystalline arrangement* of the individual polymer chains. The chemical composition of cellulose is simple: It is a homopolymer consisting of glucose monomers linked β-1,4 (Fig. 1).

To be defined as cellulose, the molecular weight of the glucan chains must be at least 30–40 kDa (i.e., the polymer must contain at least several hundred glucose residues). β-1,4-Glucans with a degree of polymerization greater than 6–8 will not remain in solution. The mechanism of glucan chain aggregation into the "insoluble" product is of great importance in understanding the diverse physical properties of cellulose. On one extreme, glucan chain aggregations result in a highly crystalline