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CHOROSELECTIVE ENZYMATIC POLYMERIZATION FOR SYNTHESIS OF NATURAL POLYSACCHARIDES

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ABSTRACT

By employing an enzymatic polymerization of activated glycosyl monomers like glycosyl fluorides and a sugar oxazoline derivative, natural polysaccharides like cellulose, xylan, and chitin, have successfully been synthesized. Regio and stereochemistry of these reactions was perfectly controlled by the action of enzyme catalysts. Further study on the synthetic cellulose formation brought about a new concept "choroselectivity" concerning a spacial (three dimensional) direction control in ordering of macromolecular chains during the polymerization.

INTRODUCTION

Nucleic acids, proteins, and polysaccharides are three important families of naturally occurring macromolecules. The functions of these macromolecules can be realized effectively provided that their macro and microconformations are strictly fixed in space in living systems. It is very important for these biopolymers to be located three dimensionally where an intermolecular polymer-polymer interaction becomes crucial in addition to intramolecular interactions derived from absolute configuration and sequence of monomer unit.

The intermolecular interactions play an important role in materials science. A typical example can be found in the crystal structure of natural polysaccharides such as cellulose and chitin where non-covalent intermolecular interactions through hydrogen bonding determine the physical property of cellulose and chitin. Polysaccharides normally have direction along the axis of the polymer chain, namely, direction defined by the reducing end and the non-reducing end. Therefore, if the relationship of a single glucan chain with neighboring glucan chain is considered, a parallel or antiparallel directional relationship due to the non-covalent interactions comes into existence.

Cellulose has two allomorphs, thermodynamically metastable cellulose I with parallel orientation and stable cellulose II with antiparallel orientation [1]. Such a relationship concerning the relative orientation of glucan chains can also be observed in case of chitin, an another important naturally occurring polysaccharide [2]. Metastable chitin with parallel orientation is called β-chitin soluble in formic acid and only small amount of such chitin can be isolated from natural systems like squid pens. Thermodynamically stable chitin with antiparallel orientation is called α–chitin insoluble in almost all solvents. It is well known that once the metastable cellulose I or β-chitin is transformed to the stable cellulose II or α–chitin, it is impossible to regenerate the initial forms. Theoretically, therefore, it is not probable to design a synthetic plan via the following two steps: preparation of more stable allomorphs (cellulose II or α–chitin) and conversion of the resulting allomorphs to the metastable ones (cellulose I or β-chitin).

Ring opening polymerization of anhydrosugars catalyzed by a Lewis acid is a useful method for polysaccharide synthesis [3]. However, this method requires appropriate protecting groups in order to achieve regioselectivity and the protecting groups must be removed at the final stage of the synthesis, producing unprotected polysaccharide itself. According to this method, it is extremely difficult to produce a metastable crystal because the final product of the unprotected polysaccharide forms as precipitates from a solution of the protected polysaccharide where a single glucan chain orients randomly each other. A new direct methodology for construction of the unprotected polysaccharide without using any protecting groups is strongly demanded to achieve the formation of a metastable crystalline. The enzymatic polymerization [4] will give us a clue to this subject.

Enzymatic Polymerization of a Sugar Fluoride Monomer Without Using Protecting Group

A hydrolytic enzyme-catalyzed polycondensation of a monomer without protecting group is expected to produce directly a polysaccharide, suggesting the *in*

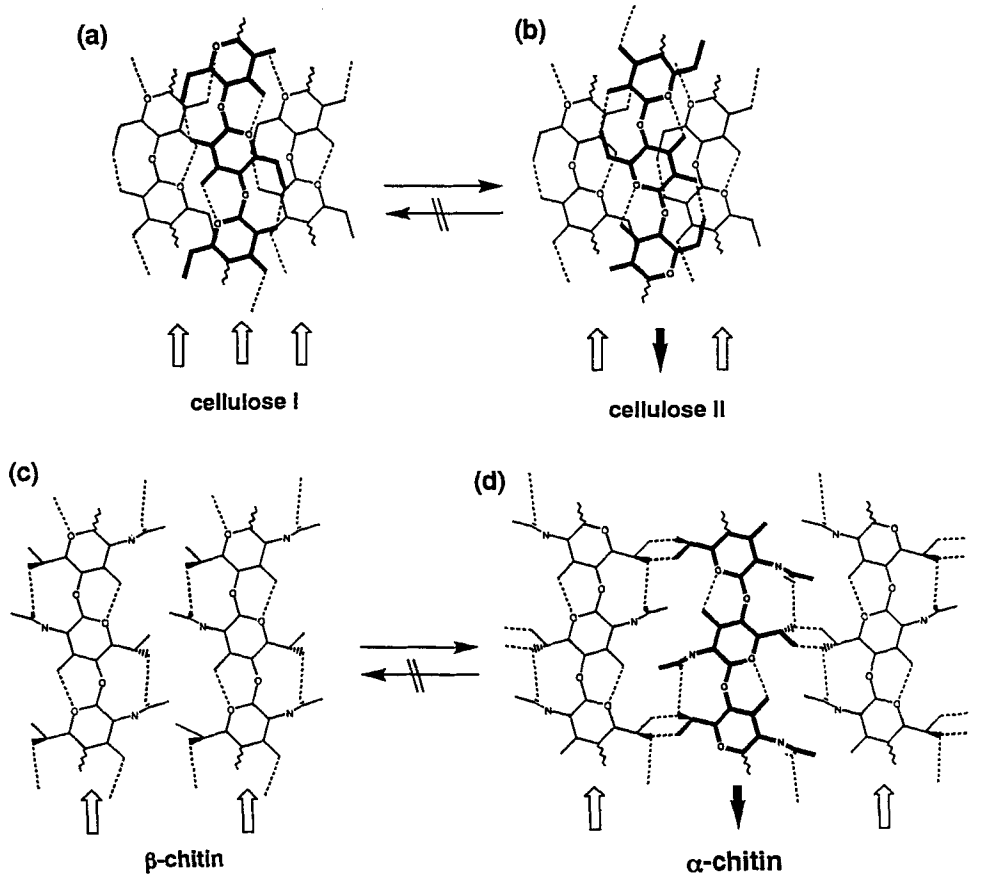
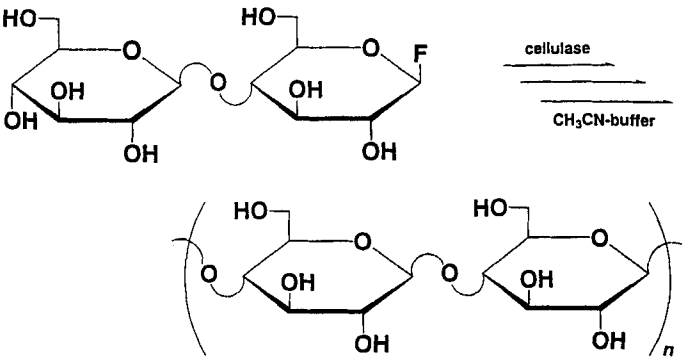


Figure 1. Relative orientation of glucan chain for typical crystalline polysaccharides, cellulose and chitin. (a) cellulose I with parallel orientation. (b) cellulose II with antiparallel orientation. (c) β-chitin with parallel orientation. (d) α–chitin with antiparallel orientation. Note the irreversible transformation from metastable cellulose I or β–chitin to more stable cellulose II or α–chitin.

vitro formation of the polysaccharide with a metastable crystalline. To promote the polymerization effectively, the selection of an appropriate leaving group at the anomeric position is an important factor. In general, halogens are known to be a good leaving group in organic reactions. However, among the glycosyl halides, only glycosyl fluoride can exist as an unprotected form; other glycosyl halides are hard to be isolated as a stable compound.

We have already reported the first *in vitro* synthesis of cellulose via a nonbiosynthetic pathway by enzymatic polymerization of ß-cellobiosyl fluoride



Scheme 1

catalyzed by cellulase, a hydrolytic enzyme of cellulose [5]. The polymerization was carried out in a mixed solvent of acetonitrile and acetate buffer in order to promote the reaction to the direction of product polysaccharide. The regio and stereochemistry between 1 and 4 position of each monomer was found perfectly controlled, leading to the ß(1→4) glucan chain (synthetic cellulose).

Crystal Structure of Synthetic Cellulose

By employing the enzymatic polymerization of ß-cellobiosyl fluoride, the elongation of a single glucan chain and the subsequent crystallization can be achieved in the same reaction system, providing an opportunity to observe the process of synthetic cellulose formation. The direct visualization of synthetic cellulose has been made by means of transmission electron microscopy and the crystal structure was further determined as the stable cellulose II by electron diffraction [6]. It was also found that the cellulase employed contains of several hundreds of proteins in addition to the cellulase component responsible for the polymerization. To our surprise, when the cellulase mixture was purified via several processes and the resulting purified cellulase was used for the polymerization of ß-cellobiosyl fluorides, a synthetic cellulose with a fibrillar morphology was detected. The electron diffraction of the fibril clearly showed the formation of metastable cellulose I allomorph in spite of the fact that its *in vitro* formation has long been believed impossible [7].

Choroselective Polymerization

Selectivities in synthetic organic chemistry can be classified into chemo-

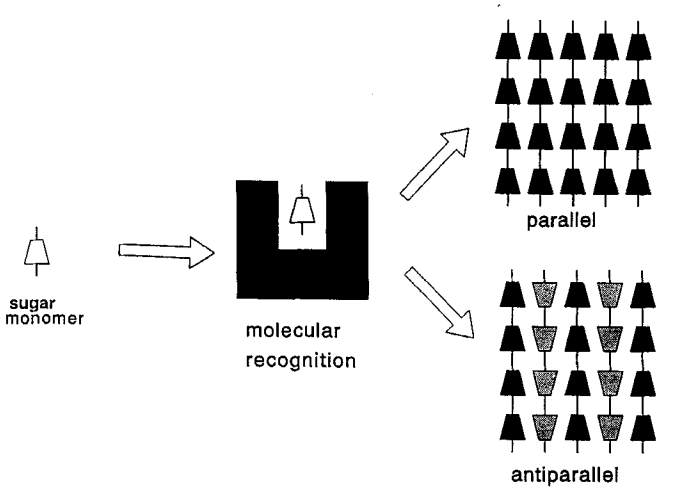
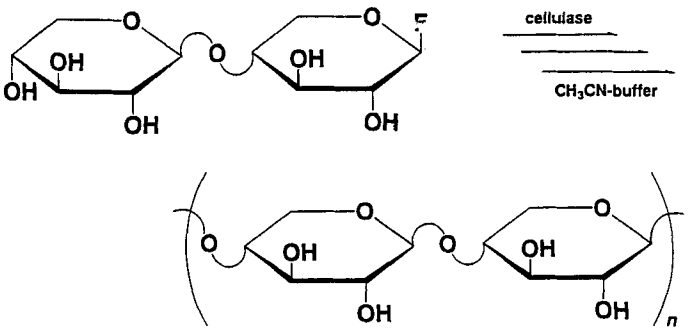


Figure 2. Formation of two allomorphs with parallel or antiparallel orientation by enzymatic polymerization of a sugar monomer. A choroselective polymerization is defined by a preferential formation of one of allomorphs during the process of polymerization.

selectivity, regioselectivity and stereoselectivity. The definition of these terms is based on a reaction which involves a covalent bond formation. The selective formation of parallel glucan chains (cellulose I) and anti-parallel glucan chains (cellulose II) concerns a non-covalent interaction and does not belong to any categories of the above selectivities. In order to express this selectivity, we proposed a new concept "choroselectivity" [8]. If polymer chain assembly showing one preferential direction of the allomorph over the other occurs during the process of polymerization, the reaction is defined as "choroselective". The term "choros" has its origin in a Greek word "χ ϖ ρ ο *s*" which means "space". This new term is widely applicable to all polymerization reactions which produce polymer chains with direction.

So far, almost all of the direction control of polymers have been accomplished by re-orientating the polymers already prepared. The typical example is the orientation of polymers under an electric field and under high pressure. Some polymers can also be oriented by utilizing their hydrophilic-lipophilic interactions or by utilizing epitaxial crystallization. Consequently, there have been no reports of choroselective polymerization; direction control at the polymerization process, starting from a monomeric molecule, where the selective orientation of polymer chains



Scheme 2

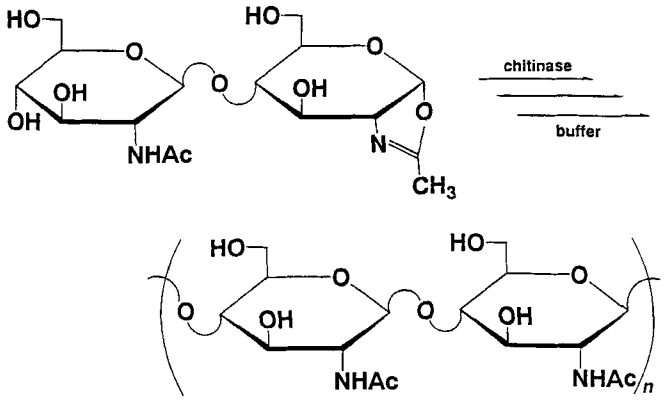
is achieved during the propagating process. Figure 2 gives an illustrative sketch of the situation.

Synthesis of Artificial Xylan

The enzymatic synthesis of cellulose suggested that the polycondensation reaction of a glycosyl fluoride catalyzed by a hydrolytic enzyme in an organic-water mixed solvent provides a general efficient method for the construction of stereoregular oligo and polysaccharides. Thus, the synthesis of xylan by the enzymatic polycondensation of ß-xylobiosyl fluoride catalyzed by a cellulase mixture (*T. viride*) has been achieved [9]. Normally, naturally occurring xylan contains several minor sugar units such as L-arabinose and 4-*O*-methylglucuronic acid in the main chain. According to the present method by using enzymatic polymerization of ß-xylobiosyl fluoride, it is possible to synthesize a xylose polymer which consists exclusively of xylose unit in the main chain.

Quantitative Chitin Synthesis by Enzymatic Polymerization of a Sugar Oxazoline Derivative

Chitin, ß(1→4)-linked poly(*N*-acetylglucosamine), has various useful properties such as antibacterial activity, wound-healing activity, and biodegradability. The first *in vitro* synthesis of chitin via a non-biosynthetic pathway has been achieved by a ring-opening polyaddition of an oxazoline derivative of chitobiose catalyzed by a chitinase, a hydrolytic enzyme of chitin [10]. The yield of the product chitin reached almost quantitative within 60 hours and, very interestingly, the yield never decreased at longer reaction times. The use of a distorted activated monomer



Scheme 3

allows the reaction to proceed in the direction of polymerization while suppressing the hydrolysis of the product chitin. The X-ray diffraction measurement of the artificial chitin showed typical peaks which are characteristic to stable α–chitin with antiparallel glucan chain orientation, which can readily be distinguishable from metastable ß-chitin.

CONCLUSION

The utility of enzymatic polymerization of activated glycosyl monomer, glycosyl fluorides and a sugar oxazoline derivative, catalyzed by glycosidases has been demonstrated. Various b(1→4) glucans, cellulose, xylan, and chitin, have successfully been synthesized *in vitro* for the first time. From the viewpoint of selectivity, these reactions proceed with perfect control of regio and stereochemistry concerning the glycosidic bond formation between each glycosyl monomer. The enzymatic polymerization was found useful not only to achieve the regio and stereoselectivities but also to control the suprastructure of cellulose; the first example of *in vitro* choroselective polymerization was shown in the cellulose synthesis. Further studies for the choroselective synthesis of b-chitin is now in progress by investigating various reaction conditions for the enzymatic polyaddition reaction of the sugar oxazoline monomer.

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