KE 1 - In Vitro Synthesis of Chitin and Chitin Derivatives via Enzymatic Polymerization

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All naturally occurring biomacromolecules are produced by in vivo enzymatic catalysis. These enzymatic reactions involve many advantages. By utilizing these enzyme-characteristics, we have been developed the in vitro enzyme-catalyzed synthesis of polymers (enzymatic polymerization) as a new method of polymer synthesis for almost these two dacades. Enzymatic polymerization enabled us to synthesize various polymers with a precisely controlled, desired structure at the molecular level. So far, a variety of poly(aromatics), polyesters, and polysaccharides have been synthesized for the first time.

In the present paper, we demonstrate the first in vitro synthesis of natural chitin and unnatural chitin derivatives via this method.

Chitin is a polysaccharide of N-acetyl-D-glucosamine connecting through β(1→4) fashion. The hydrolysis mechanism of chitinase, which catalyzes hydrolysis of β(1→4) glycosidic linkage in chitin, is postulated as that bond-cleavage occurs concurrently with the formation of an oxazolinium ion intermediate. Therefore, we designed N,N-diacetylchitobiose oxazoline derivative (Chi-oxa) as a transition-state analogue substrate (TSAS) monomer for chitinase catalysis. Chi-oxa was recognized by chitinase, leading to synthetic chitin via a ring-opening polyaddition.

Taking advantages of dynamic nature of enzyme, enzymatic polymerization has been applied to synthesis of unnatural polysaccharides. Synthesis of a 6-fluorinated chitin derivative was achieved by chitinase-catalyzed ring-opening polyaddition of a fluorinated Chi-oxa as a TSAS monomer.

Hybrid-type polysaccharides were produced using a monomer consisting of different sugar moieties. A chitin/cellulose hybrid polysaccharide was synthesized with two-kinds of reaction; via ring-opening polyaddition of the corresponding oxazoline-type TSAS monomer by chitinase catalysis and via polycondensation of a fluoride-type TSAS monomer by cellulase catalysis. A chitin/xylan hybrid polysaccharide was produced by chitinase-catalyzed polymerization of an oxazoline-type monomer.

For the synthesis of chitosan derivative, we newly designed and synthesized an N-acetylchitobiose oxazoline derivative as TSAS monomer for chitinase catalysis. The monomer was recognized by chitinase and polymerized via ring-opening addition to give a water-soluble alternatingly de-N-acetylated chitin derivative, a chitin/chitosan hybrid polysaccharide.