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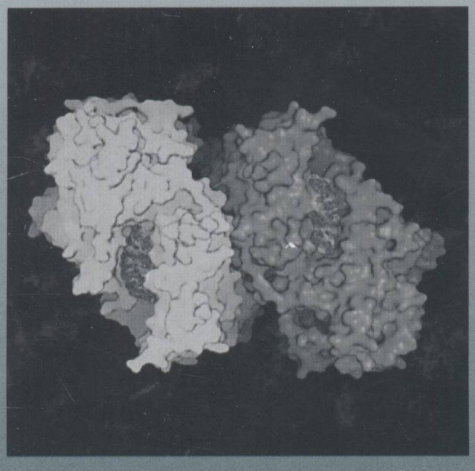

Carbohydrate-active enzymes

Structure, function and applications

Edited by Kwan-Hwa Park

CRC

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PREFACE

Recent advances in biochemistry and biotechnology have provided significant progress on basic research and applications of carbohydrate active enzymes. However, the mechanism of the catalytic reaction has not been fully understood, as the enzymes often showed unusual substrate specificity and mode of action. Therefore, this symposium has emphasized the enzymatic reaction mechanism, structure–function relationship and role in the living organism. The Center for Agricultural Biotechnology (CAB) at Seoul National University has organized the "Annual Symposia on Agricultural Biotechnology" including carbohydrate enzymes since 1990. Out of this symposium, a number of excellent results on the new types of carbohydrate enzymes and their applications have been reported. This volume, "Carbohydrate-active enzymes: structure, function, and application", based on the 2008 Agricultural Biotechnology Symposium in Seoul, September 26–27, 2008 organized by CAB, summarizes the current information on carbohydrate enzymes by international experts. This book has primarily focused on the classification, structure, specific mechanisms of amylolytic enzymes, metabolism and applications in the hope that it will stimulate the readers and drive work for future research. We hope that readers will find this book useful for the current status of some carbohydrate enzymes that have not been well investigated.

We would like to thank all the authors for their magnificent work, time and devotion. I am grateful to Professor In-Won Lee, former director of CAB who initiated this symposium specifically on carbohydrate enzymes and led the organizing committee and editorial members to publish this book. I would also like to thank Professor Tae Wha Moon, director of CAB and other members of CAB for their continuous support. My special thanks are extended to Professors Sang-Ho Yoo, Suyong Lee, Myo Jeong Kim, Young Wan Kim, Hee Seop Lee, Young Jin Choi for editorial efforts. I am grateful to my co-workers, who have contributed to this book in various ways in particular Professor Pan Sik Chang and Professor Yong-Ro Kim.

Kwan-Hwa Park

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Part II Functions and applications of carbohydrate-active enzymes

PART I

STRUCTURE–FUNCTION RELATIONSHIP OF CARBOHYDRATE-ACTIVE ENZYMES

BIOSYNTHESIS OF POLYSACCHARIDES

John F. Robyt

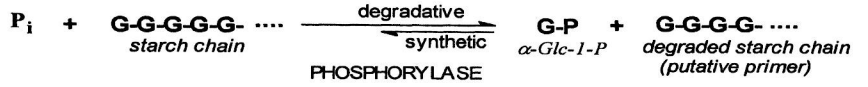
ABSTRACT

The mechanisms involved in the biosynthesis of six polysaccharides is described in the following order: (1) Introduction to the first purported biosynthesis of polysaccharides, glycogen and starch by phosphorylases; (2) biosynthesis of *Salmonella* O-antigen polysaccharide; (3) biosynthesis of bacterial cell wall polysaccharide, peptido-murein; (4) biosynthesis of dextran by B-512FMC dextransucrase; (5) biosynthesis of bacterial cellulose and xanthan; (6) biosynthesis of starch in starch granules. The structures of the six polysaccharides are quite diverse. There are four β-linked hetero-polysaccharides (2), (3), and (5), and two α-linked homo-polysaccharides (4) and (6). The first five are biosynthesized by prokaryote bacteria and the sixth polysaccharide (starch) was shown to be biosynthesized by eight different eukaryotic plant sources. All six of the polysaccharides have been shown to be biosynthesized by a common mechanism in which the monomer or repeating unit is added to the reducing-end of a growing polysaccharide chain in a two catalytic-site insertion mechanism. The β-linked polysaccharides are covalently α-linked to a lipid pyrophosphate, bactoprenol pyrophosphate, at the active-site of the synthesizing enzymes; the α-linked polysaccharides are β-linked directly to the synthesizing enzymes. When the monomer or repeating unit is inserted between the growing polysaccharide and the lipid pyrophosphate or the enzyme, the configuration of the linkage of the polysaccharide is inverted, giving the correct stereochemistry for the specific polysaccharide. Eventually, the polysaccharides are released from the active-sites by an acceptor reaction with water or with another carbohydrate.

Key words: cellulose synthase; dextransucrase; insertion mechanism; primer mechanism; starch synthase

INTRODUCTION

Polysaccharides were the first biopolymers purported to be biosynthesized *in vitro* (Cori and Cori 1939) observed that the reaction of liver phosphorylase with α-D-glucose-1-phosphate (α-Glc-1-P) and glycogen added glucose residues to the nonreducing-ends of glycogen chains. Shortly thereafter, Hanes (1940) reported a similar reaction for potato phosphorylase in which α-Glc-1-P and starch also added glucose residues to the nonreducing-ends of the starch chains. Up to this time, the reaction catalyzed by phosphorylases was with inorganic phosphate (P_i) and glycogen or starch chains to give α-Glc-1-P and a partially degraded polysaccharide. It was found that phosphorylases catalyzed these two reactions with equilibrium constants close to one (Swanson and Cori, 1948). The equilibrium, however, seemed to favor the degradation reaction than the synthetic reaction. The reactions were formulated for glycogen and starch chains, as the following:



The reactions show that the degradation involves inorganic phosphate that removes glucose residues from the nonreducing-end of the polysaccharide chains to remove glucose residues and form α-Glc-1-P and a partially degraded polysaccharide chain. The reverse, synthetic reaction, involves the transfer of glucose from α-Glc-1-P to α-1→4 glucan chains or to the nonreducing-ends of an α-1→4 linked glucose oligosaccharide. The addition of just α-Glc-1-P to the phosphorylases, however, gave no reaction. It was, thus, recognized that a preformed polysaccharide or oligosaccharide chain was absolutely required to have synthesis by these reactions and the concept of a *required primer* was established.

As the reaction was studied more carefully, it was found that starting with α-Glc-1-P and a starch or glycogen chain, the reaction rapidly slowed down and stopped, as the concentration of P_i increased. It was further found that the synthetic reaction did not occur *in vivo* at all, as the concentration of P_i in animal and plant tissue was 20- to 40-times the concentration of α-Glc-1-P (Trevelyan et al., 1952; Ewart et al,. 1954; Liu and Shannon, 1981) and the *in vivo* conditions greatly favored degradation, rather than synthesis. Further, the addition of phosphorylases to just α-Glc-1-P gave no reaction. It, thus, appeared that phosphorylases only catalyzed the degradation of glycogen and starch and not the synthesis.

The studies of (Cori and Cori, 1939; Hanes, 1940; and Swanson and Cori, 1948), however, led to the development of the hypothesis for a *required primer* chain for the biosynthesis of polysaccharides. With essentially no evidence this concept has stuck in the minds of many people since then and relatively recently, it has been postulated for the mechanism of biosynthesis of polysaccharides, even with a paucity of experimental evidence (Bocca et al., 1997; Ball et al. 1998; Ball and Morell, 2003; and Tomlinson and Denyer, 2003).

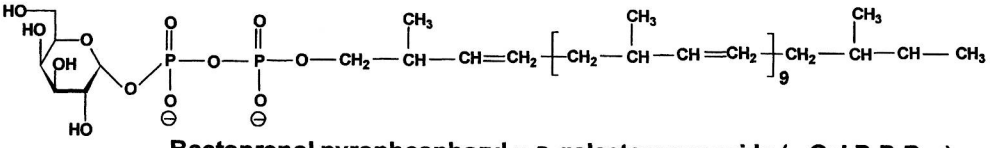
Some 20 years after the phosphorylase experiments, (De Fekete et al., 1960; Recondo and Leloir, 1961; Leloir et al., 1961) found that the high-energy donor of glucose for starch biosynthesis was uridine diphospho glucose (UDPGlc) and adenosine diphospho glucose (ADPGlc) and that when ADPGlc was incubated with starch granules, starch chains were biosynthesized. ADPGlc was the better of the two donors. The biosynthetic enzymes, *starch synthase* and *starch branching enzyme* were apparently entrapped in the granules during their synthesis. Many years later, (Robyt and Mukerjea, 2000) found that starch granules that had been in bottles on the laboratory shelves for over 40 years, still retained the ability to incorporate glucose from ADPGlc into starch.

When De Fekete et al. (1960), Recondo and Leloir (1961), and Leloir et al. (1961) incubated starch granules with ADP-[^{14}C] Glc, ^{14}C-glucose was incorporated into the starch. When they solubilized the starch and reacted it with the exo-acting enzyme, β-amylase, they obtained ^{14}C-labeled maltose from which they assumed that the synthesis of starch involved the addition of glucose from ADPGlc to the nonreducing-ends of the starch chains. This experiment has been widely considered as proof that starch chains are biosynthesized by the addition of glucose from ADPGlc to the nonreducing-ends of starch primer chains. This assumption, however, is not necessarily correct in that if the starch chains had been synthesized *de novo* from the reducing-end, rather than from the nonreducing-end of a primer, the synthesized chains would have every

glucose residue in the chains labeled, and the subsequent reaction with β-amylase would also give ^{14}C-labeled maltose. See Section 6 for recent studies on how starch is biosynthesized.

MECHANISM FOR THE BIOSYNTHESIS OF *SALMONELLA* O-ANTIGEN POLYSACCHARIDE

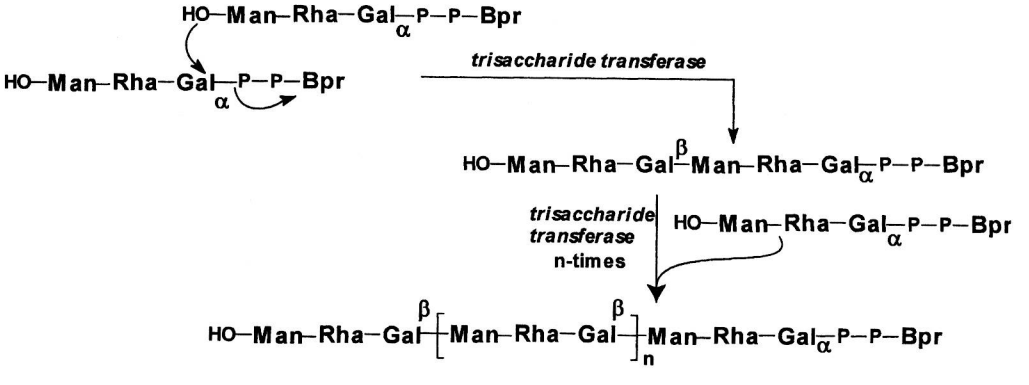
The O-antigen surface polysaccharide of *Salmonella anatum* is a hetero-polysaccharide that was the first polysaccharide to have its mechanism of synthesis definitively determined (Dankert, et al. 1966; Wright et al., 1967; Bray and Robbins, 1967; Robbins et al., 1967). The polysaccharide is composed of a linear structure of β-mannosyl-β-rhamnosyl-β-galactosyl repeating sequence. The trisaccharide is biosynthesized from the sugar diphospho nucleotides, GDPMan, TDPRha, and UDPGal. The first reaction is the reaction of UDPGal with a lipid phosphate, bactoprenol phosphate to give bactoprenol pyrophosphoryl-α-D-galactopyranoside (Dankert et al., 1966; Wright et al., 1967)



Bactoprenol pyrophosphoryl α-D-galactopyranoside (α-Gal-P-P-Bpr)

Assembly of the trisaccharide then occurs by the enzyme catalyzed addition of L-rhamnose to C4-OH of D-galactose, and the addition of D-mannose from GDPMan to the C4-OH of L-rhamnose to give Man–Rha–Gal–P–P–Bpr. This trisaccharide bactoprenol pyrophosphate is synthesized inside the cell by the addition of the monosaccharides in sequence to the bactoprenol pyrophosphate, which is partially embedded in the lipid bilayer of the cell membrane. The trisaccharide is enveloped by bactoprenol and is then transported through the lipid membrane to the outside of the cell, where polymerization occurs.

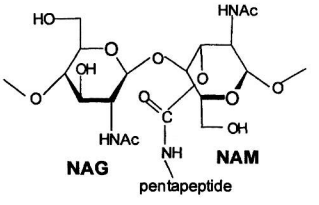
Bray and Robbins (1967) showed, by pulse and chase experiments, that the repeating trisaccharide was transferred to the reducing end of a growing chain according to the following reactions:



The C4-OH of the D-mannose makes a nucleophilic attack onto the C1 of the D-galactose, giving inversion of the configuration from α to β and the insertion of the trisaccharide between the reducing-end and the bactoprenol pyrophosphate. This reaction occurs repeatedly to give polymerization of the polysaccharide by the addition to the reducing-end.

MECHANISM FOR THE BIOSYNTHESIS OF BACTERIAL CELL WALL POLYSACCHARIDE, MUREIN

Murein is a polysaccharide with a repeating sequence of N-acetyl-D-glucosamine (NAG) linked β-1→4 to N-acetyl-D-muramic acid (NAM) in which a pentapeptide is attached to the carboxyl group of NAM. It also was found that bactoprenol phosphate was involved in the biosynthesis of the bacterial cell wall poly-peptidomurein (Anderson, et al., 1965; Struve and Neuhaus, 1965; Struve et al., 1966):



NAG-NAM-pentapeptide repeating unit of bacterial cell wall polysaccharide

The biosynthesis also starts inside the bacterial cell, where UDP-N-acetyl-D-muramic acid reacts with bactoprenol phosphate to give α-N-acetyl-D-muramic acid pentapeptide bactoprenol pyrophosphate plus UMP. N-Acetyl-D-glucosamine is then enzymatically added to C4-OH of the N-acetyl muramic acid in a β-linkage to give NAG-NAM-bactoprenol pyrophosphate, which is then transported through the cell membrane lipid bilayer to the outside of the cell where it is polymerized. Using ^{14}C-N-acetyl-D-glucosamine, it was reported in 1973 that the disaccharide is added to the reducing-end of a growing murein chain by the C4-OH of NAG attacking C1 of NAM at the reducing-end of the growing chain, giving the insertion of the disaccharide between the growing chain and the bactoprenol pyrophosphate (Ward and Perkins, 1973), essentially an identical mechanism, as the biosynthesis of *Salmonella* O-antigen polysaccharide: