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Review Paper

Polysaccharide Research in Trondheim

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ABSTRACT

Current research on polysaccharides in Trondheim is summarized, with emphasis on the interests and activities of the individuals concerned. The present research activity has evolved mainly from early work on seaweeds and polysaccharides derived from them. Polysaccharides produced by unicellular and other planktonic algae were later added to the list of interests, and this has now expanded to include bacterial exopolysaccharides of special industrial significance, capsular proteoglycans of edaphic algae, and polysaccharides from mosses. The emphasis ranges from fundamental aspects of aquatic and edaphic ecology to practical problems such as biofilm formation. The preparation and use of well defined samples and development of new or improved instrumental methods of characterization of polysaccharides are other special features. The Norwegian Biopolymer Laboratory (NOBIPOL) is a new organization which functions within the University, initiating and coordinating multidisciplinary research programmes centred on polysaccharides.

INTRODUCTION

Polysaccharide research in Trondheim is inevitably linked to the Institute of Biotechnology at the Norwegian Institute of Technology. This Institute now incorporates the former Institute of Marine Biochemistry, which before 1973 was known as the Norwegian Institute of Seaweed Research. Current research on polysaccharides has therefore developed

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from fundamental and classical work on seaweeds and polysaccharides derived from seaweeds. Research on alginates and other phycocolloids are still major activities, but close cooperation with other research groups has added new dimensions to polysaccharide research in Trondheim. Cooperation with the biophysical instrumentation group at the Institute of Physics has been especially fruitful, and a group within UNIGEN (Center for Molecular Biology, University of Trondheim) are increasing their research efforts on polysaccharide engineering.

In 1988, the Norwegian Biopolymer Laboratory (NOBIPOL) became an official institution within the University of Trondheim. Directed by Olav Smidsrød (Institute of Biotechnology) and Arnljot Elgsæter (Institute of Physics), NOBIPOL initiates and coordinates multi-disciplinary research programmes related to biopolymers.

This article outlines recent and current research activities related to polysaccharides by focusing on ongoing projects, and the persons and special methods connected with each project. For detailed descriptions of important projects, reference will be made to some recent scientific papers. Figure 1 and Table 1 provide an overview of most of the staff involved in polysaccharide research in Trondheim. Table 2 lists the polysaccharides currently studied.

THE TRONDHEIM APPROACH TO POLYSACCHARIDE RESEARCH

Research on polysaccharides in Trondheim has been concerned mostly with structure–function relationships. The performance of precise physical measurements upon well-defined, homogeneous samples has been the key to progress in many cases. This has meant that samples had to be produced, purified and analysed in our own laboratory, because commercial samples were too poorly defined. For copolymers like alginate, and more recently chitosan, this approach has proved to be vital. It is also well adapted for our more recent research based on advanced instrumentation. Again, carefully characterized samples are examined by specialized physical methods, in which the instrumentation in some cases has been developed specifically for the samples and the scientific problems to be solved. This is in contrast to the more usual approach, in which samples and problems are selected so that they can be studied with existing commercial equipment.

Alginates — structure and properties

Following classical work in the 1950s and 1960s directed by the late Arne Haug, more than 70 scientific papers related to alginate have been

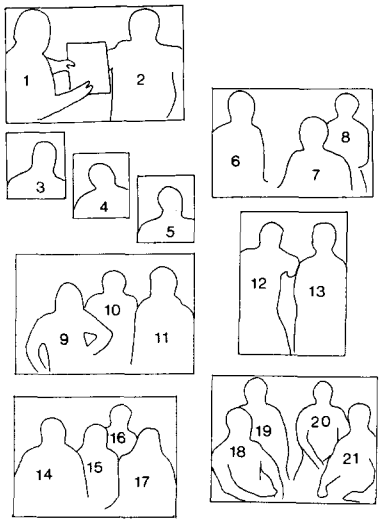


Fig. 1. Some key persons involved in polysaccharide research in Trondheim. Olav Smidsrød (1), Arnljot Elgsæter (2), Sverre Myklestad (3), Terence Painter (4), Bjørn Larsen (5), Bjørn T. Stokke (6), Arne Mikkelsen (7), Kenneth Knudsen (8), Sissel Hertzberg (9), Kjetill Østgaard (10), Kurt I. Draget (11), Bjørn E. Christensen (12), Kjell M. Vårum (13), Gudmund Skjåk-Bræk (14), Anita Martinsen (15), Størker Moe (16), Wenche Strand (17), Hans Grasdalen (18), Svein Knutsen (19), David Myslabodski (20) and Ole E. Bakøy (21).

TABLE 1
Main Activities of Most of the Staff Involved

At the Institute of Biotechnology	
Hans Grasdalen	Primary structure, conformation and dynamics of polysaccharides in solutions and gels
	NMR methods
Bjørn Larsen	Biosynthesis of polysaccharides
	Immunoassays
Sverre Myklestad	Structure, properties and ecological functions of β-glucans from diatoms
Terence Painter	Structure–function relationships, evolutionary aspects and industrial and agricultural applications of polysaccharides from algae and mosses
	New chemical methods for structural studies
	Selective oxidation with nitrous acid and periodate
	Project leader on polysaccharides in desert reclamation
Olav Smidsrød	Structure–function relationships and industrial applications
	Director of NOBIPOL
	Leader of national research programme 'Industrial applications of biopolymers'
Ole E. Bakøy	Pectins: sequence studies by NMR
	Alginate–pectin synergism (with H. Grasdalen)
Bjørn E. Christensen	Chemical and physical stability of polysaccharides
	Role of polysaccharides in biofilms
	Coordinator of national research programme 'Industrial applications of biopolymers'
Sissel Hertzberg	Biocatalysts in organic solvents
	Immobilization in alginate
Svein Knutsen	Sulphate distribution in seaweed galactans (with H. Grasdalen)
Anita Martinsen	Alginate gel beads, physical and diffusional properties (with O. Smidsrød and G. Skjåk-Bræk)
Størker Moe	Chemically cross-linked alginate gel beads (with O. Smidsrød and G. Skjåk-Bræk)
David Myslabodski	Evaluation of extraction parameters for agar (with B. Larsen)
Svein Ramstad	Fermentation of polysaccharides (with David Levine)
Gudmund Skjåk-Bræk	Structure–function relationships in alginates related to immobilization
	Alginate biosynthesis
Kjell M. Vårum	Structure–function relationships of β-glucans and chitosan (with O. Smidsrød)
Kjetill Østgaard	Protoplast, cell and tissue culture of seaweeds
	Alginate biosynthesis in protoplasts of brown algae
At the Institute of Physics	
Arnljot Elgsæter	Biophysical instrumentation
	Rheology and dynamics of polysaccharides
	Director of NOBIPOL

TABLE 1 — *contd.*

Kenneth Knudsen	Spectropolarimetry and viscometry at elevated temperature and pressure
Arne Mikkelsen	Elongational-flow birefringence (instrumentation)
Bjørn T. Stokke	Electron microscopy Rheology Conformational calculations of polysaccharides
At the Institute of Botany	
Kurt I. Draget	Homogenous alginate gels Immobilization and micropropagation of plant cells and protoplasts (with K. Østgaard)
At the Institute of Cancer Research, Regional Hospital	
Marit Otterlei	Immunostimulating and toxic effects of alginates (with T. Espevik, G. Skjåk-Bræk and O. Smidsrød)
At UNIGEN (Centre for Molecular Biology)	
Svein Valla	Biosynthesis of bacterial cellulose Cloning of mannurono-C5-epimerase
Dag H. Coucheron	Biosynthesis of bacterial cellulose Cloning of genes in the biosynthesis of xylinan
Tore-Geir Iversen	Cloning of genes in the biosynthesis of xylinan (Extracellular polysaccharide of *Acetobacter xylinum*)

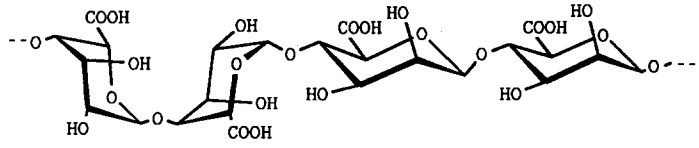
published by the Institute of Biotechnology. Major breakthroughs include the discovery of the blockwise distribution of the two monomeric components of alginate, D-mannuronic acid (M) and its 5-epimer L-guluronic acid (G) (Fig. 2). This, together with studies of the unique mechanism of epimerization of M into G at the polymer level, the mechanism of gelation (Fig. 3), the ion-binding properties, and solution properties, has provided us with an exceptionally well-understood polysaccharide system with unique properties and a growing number of industrial applications. Alginate research is still a dominating, even a growing, activity. Recent work, which includes isolation, characterization, cloning and technical use of the key enzyme mannurono-C5-epimerase, adds new dimensions, even at the present stage.

The structural characterization of alginate in the early days relied on chemical methods, which were tedious, even though they were brought close to perfection. However, the introduction of modern NMR methods opened up a new dimension. Hans Grasdalen and co-workers have developed a range of techniques based on NMR which are now used

TABLE 2
Polysaccharides Currently Studied in Trondheim

Type	*Source*	*Relevance*
Alginate	Seaweeds, bacteria	Gels, gel beads and capsules
Xanthan	Bacteria[a]	Viscosifier, gels
Scleroglucan	Fungus[a]	Viscosifier
Gellan (S-60)	Bacteria[a]	Gelling agent
S-series	Bacteria	Model substances
PS-A/B/C	Bacteria (marine)	Bacterial adhesion, biofilms
β-glucans	Cereals	Dietary fibre
	Marine microalgae (plankton)	Vaccines (laminarin)
Carrageenan	Red algae	Gelling agent, food additive
Agar	Red algae	Gelling agent, food additive
Pectin	Plant cell walls[a]	Gelling agent, viscosifier, food additive
Sphagnum holocellulose	Peat moss	Cation exchange resins, immobilization of enzymes
Chitosan	Shrimp, crab	Capsules, water purification, medical uses
EPS cyanobacteria	Cyanobacteria	Water retention (desert reclamation)
Xylinan	Mutant of *Acetobacter xylinum*	Model polysaccharide for genetic engineering and large-scale fermentation

[a]Commercial samples.



L-GulA-α-1,4-L-GulA-α-1,4-D-ManA-β-1,4-D-ManA

Fig. 2. Fragment of an alginate molecule — α-L-guluronic (L-GulA) acid, β-D-mannuronic aid (D-ManA).

routinely in all alginate research (Grasdalen, 1983). The gradual appearance of progressively more sophisticated high-field spectrometers has permitted the determination of, for instance, triad frequencies and the position of acetyl groups in bacterial alginates (Skjåk-Bræk *et al.*, 1986*a*). It is important to point out that proper treatment of alginate samples prior to NMR analysis is crucial for obtaining high-quality spectra.

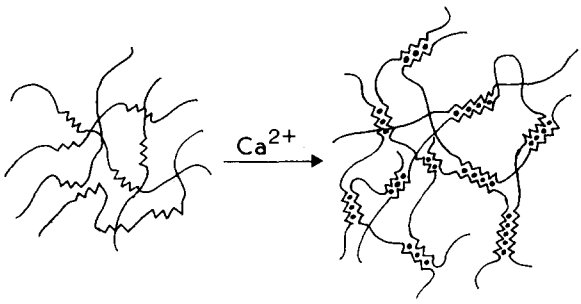


Fig. 3. Schematic representation of the calcium-induced gelation of alginate.

A useful supplement to NMR methods is the use of alginate lyases. Such enzymes, which work on various block structures with high degrees of specificity, are studied by Bjørn Larsen (Haugen *et al.*, 1989). He also makes use of monoclonal antibodies prepared against alginates with different compositions (Larsen *et al.*, 1985).

Alginate as immobilization material

The development of a new and simple device for producing alginate beads had led to new research on their use for the immobilization of microorganisms, mammalian and plant cells, and enzymes. This work is carried out in close cooperation with Norwegian and international industrial companies. We can now produce tailor-made alginate beads for a variety of purposes (Fig. 4). Gudmund Skjåk-Bræk and Anita Martinsen have recently studied the process in detail (Skjåk-Bræk *et al.*, 1986*b*; Martinsen *et al.*, 1988). Through control of the gelation kinetics the properties of the beads can be varied. For example, inhomogeneous beads with capsule-like properties can be prepared with steep concentration gradients (Skjåk-Bræk *et al.*, 1989*a*). The rheological properties of the beads, and other systems, are studied by special instrumentation (see below). Another type of bead can be prepared by chemically cross-linking alginates. Such beads display many interesting properties resembling gels of synthetic polymers. An example is super-swelling beads which may absorb 100 times their own volume of water within a few minutes. Størker Moe joins Gudmund Skjåk-Bræk in this work.

Through control of the gelation process, macroscopic and homogeneous alginate gels can now be made. In this work, Kjetill Østgaard and Kurt I. Draget have demonstrated that alginate may solidify biological media to give gels with functional properties comparable to

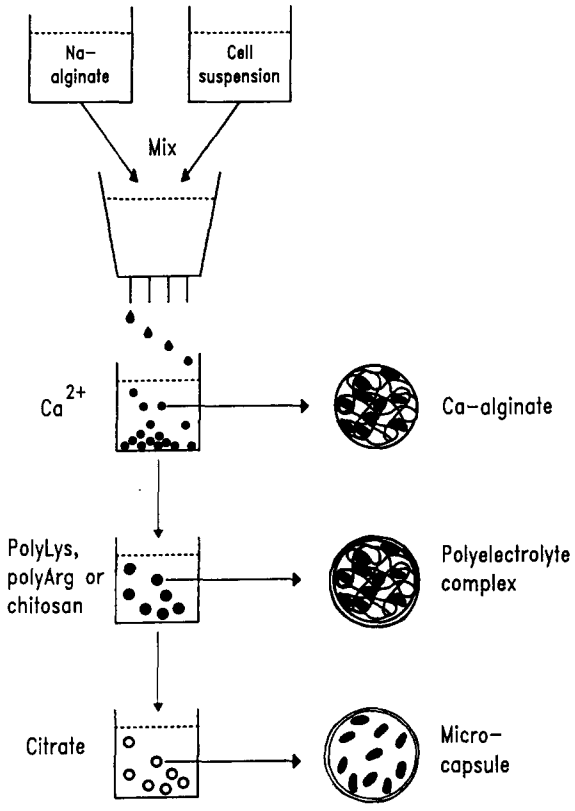


Fig. 4. Production of different types of alginate beads and capsules.

the more expensive agars and agarose. This is accomplished without a melting/cooling step at room temperature, and the resulting gels exhibit high optical clarity and gel strength. One established application of such gels is as a substitute for agar gels in plant tissue culture (Draget *et al.*, 1989). Highly efficient regeneration of plant protoplasts by immobilization in Ca-alginate beads has also been achieved (Draget *et al.*, 1988).

Sissel Hertzberg is cooperating with colleagues in several departments in studies of alginate as an immobilization agent for performing biocatalytic reactions in organic solvents, and for the immobilization of microalgae (Hertzberg & Jensen, 1989) and mammalian cells for biotechnological purposes.

Alginate gels are also potential carriers for cells, enzymes or drugs within the human body; for instance, in vaccines or for implantation purposes. This requires extremely well-controlled gel properties, the most important of which is the mediated release of the immobilized

agent, immunoglobulins, etc. Special attention is paid to the toxic and immunogenic properties of alginates and minor contaminants in alginate preparations. This field is studied in cooperation with Terje Espevik and Marit Otterlei at the Institute of Cancer Research at the Regional Hospital in Trondheim.

Biosynthesis of alginate

Alginate-producing bacteria, mainly *Azotobacter vinelandii*, have so far been used in most of our studies of alginate biosynthesis, and in particular of the enzyme mannurono-C5-epimerase (Skjåk-Bræk & Larsen, 1985). Despite its commercial importance, less is known about alginate biosynthesis in brown algae. This is due in part to the presence of phenols in the algae. Kjetill Østgaard and co-workers in Leeds (UK) and Roscoff (France) have now been able to produce protoplasts from *Laminaria* (Butler *et al.*, 1989), which offer new possibilities for studying regeneration of cell walls and, hence, alginate synthesis.

Agar and carrageenan

Despite the established use of these phycocolloids from red algae, much basic work is going on in this field, also in Trondheim. Currently, Svein Knutsen is investigating galactans from different sources using specific enzymes and NMR (Knutsen & Grasdalen, 1987), whereas David Myslabodski is working on the optimization of agar extraction. The nature of the ordered conformation of kappa-carrageenan is one of the classic controversies in polysaccharide chemistry, with diverging results and conclusions. Crucial information about the structure and the role of ions in conformational transitions and gelation has been obtained by ion-NMR techniques (Grasdalen & Smidsrød, 1981*a*, *b*). A new development is the study of the conformational behaviour of oligomeric fragments of carrageenan using 2D-NMR techniques combined with model-building in supercomputers. Hans Grasdalen coordinates this work.

Pectin

The degree of esterification and the distribution of methyl ester groups and free carboxyl groups are of critical importance for the physical, biochemical and functional properties of pectin. Hans Grasdalen and Ole E. Bakøy have characterized the esterification pattern in pectin by NMR (Grasdalen *et al.*, 1988). Such information facilitates present

studies of the mechanism of action of pectin esterases and the synergistic gelation with alginate (Toft *et al.*, 1986).

Sphagnum holocellulose

In recent work on peat mosses, Terence Painter described a holocellulose with unique properties. It is a complex heteroglycan built up of cellulosic, amyloid-like and pectin-like chains. These are covalently cross-linked into a 3D network through residues of D-*lyxo*-5-hexosulopyranuronic acid (5KMA). The combination of high selectivities in the exchange of divalent metal ions and good column-packing properties makes it an ideal material for chromatographic separation and retention of such cations (Andresen *et al.*, 1987). The holocellulose also contains reactive keto-groups, which facilitate the preparation of numerous derivatives.

β-Glucans

These polysaccharides are found in a variety of biological systems. Due to their occurrence in planktonic algae, they are second only to cellulose in terms of global production of biopolymers. Sverre Myklestad is studying the structure, biosynthesis and production of such planktonic β-glucans, using culture studies, radioactive tracer techniques and biochemical methods (Vårum *et al.*, 1986).

As components of cereals, β-glucans are classified as 'dietary fibres'. Kjell M. Vårum examines the solution properties of β-glucans from oats, using HPLC, light scattering, viscometry and osmometry (Vårum & Smidsrød, 1988).

Chitosan

Chitosan is a family of polysaccharides prepared by partial or complete deacetylation of chitin, which occurs in crustaceans. Shells of crabs and shrimps are processed on an industrial scale to yield chitosans. Despite a large and rapidly growing industrial use, there remains much basic work to be done on chitosan, as concluded at the 4th International Conference on Chitin and Chitosan which was organized by the Institute of Biotechnology in Trondheim, August 1988 (Skjåk-Bræk *et al.*, 1989*b*).

A recently initiated research activity in Trondheim is to study the role of acetyl distribution (random or blockwise) on the properties of chitosan. Olav Smidsrød and Kjell M. Vårum are organizing this work in cooperation with Hans Grasdalen and students. Methods for preparing

chitosans with different types of acetyl substitution are being studied, and NMR methods for determining the sequence of monomers (*N*-acetylglucosamine and glucosamine) are currently being developed.

Polysaccharides from film-forming bacteria

The formation of biofilms, which are microorganisms growing at interfaces, involves the production of microbial polysaccharides at several stages, although the detailed roles of the film-associated polysaccharides are unclear (Christensen, 1989). By using a marine *Pseudomonas* sp. as a model organism Bjørn E. Christensen isolated and examined two fundamentally different polysaccharides (Christensen *et al.*, 1985). One of them (PS-A), which resembles typical bacterial polysaccharides of the 'xanthan-type', may be involved in maintaining a gel-like, extracellular matrix, while the other (PS-C) is cell-bound and may influence the adhesive properties of the bacterium; the latter also displays some unusual solution properties. Current activities also include studies of the role of polysaccharides in biofilm removal (detachment), which in many ways resembles the disintegration of biopolymer gels.

Xanthan

In cooperation with the oil industry, the relationships between the conformation and properties of xanthan (Fig. 5) have been studied in

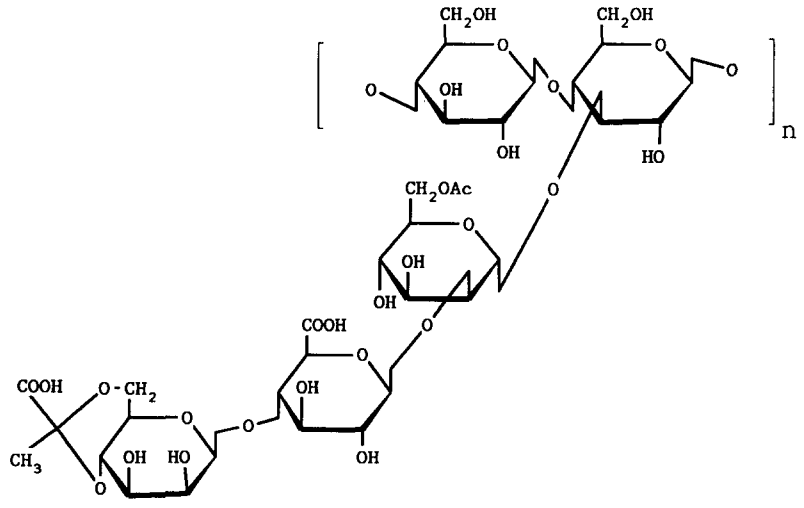


Fig. 5. Repeating pentasaccharide unit of xanthan. The amount of pyruvate diketal and *O*-acetate linked to the β-mannose and the α-mannose residues, respectively, may vary.

detail. Through this work there has been developed a new and fruitful cooperation between the biophysical instrumentation group (Institute of Physics), led by Arnljot Elgsæter, and the biopolymer group (Institute of Biotechnology), led by Olav Smidsrød.

It has been claimed that xanthan adopts a single-helical conformation in the ordered state in solution, whereas others claim that it is double-stranded. Bjørn T. Stokke, therefore, refined the preparation techniques for electron micrographs of biopolymers, and was able to demonstrate two classes of individual molecular species (Stokke *et al.*, 1986, 1989*a*). One of these was thick and stiff (estimated persistence length, 150 nm) with an apparent linear mass density of ≈ 2000 daltons nm^{-1}, and the other was flexible (estimated persistence length, 60 nm) and thinner. Most importantly, it was possible to observe the splitting of the thick strands into two of the thinner ones. This technique also provided information about microaggregates (Stokke *et al.*, 1989*a*, *b*), which strongly influence the solution properties.

The use of xanthan or other biopolymers in enhanced oil recovery requires that the solutions maintain their original viscosity for extremely long periods (years) at high temperatures, pressures and salt concentrations. The stability properties of xanthan are currently being studied by Bjørn E. Christensen. Using a double-stranded model as a working hypothesis the viscosity loss and changes in chemical composition upon induced degradation are monitored. Current data seem to support the hypothesis of a double-stranded structure.

Xanthan forms gels in the presence of several trivalent cations. The Cr(III)–xanthan gel system has recently been studied. In particular, the kinetics of gelation have been investigated in detail (Lund *et al.*, 1988). For this work, special equipment was designed and built (see below).

Polysaccharides from edaphic cyanobacteria

Some blue-green soil algae (also called cyanobacteria) are capable of nitrogen fixation, and simultaneously produce an abundance of extracellular proteoglycan with a high capacity for water retention. These organisms may be exploited as a kind of self-reproducing fertilizer and soil conditioner, and may be especially useful in the urgent struggle against desert encroachment in the Sahel region of Africa. In a joint project between Antonella Flaibani (University of Trieste, Italy), Yngvar Olsen at SINTEF (Trondheim) and Terence Painter here at the university, some selected organisms are studied closely with respect to growth, proteoglycan production and the chemical and physical properties of the proteoglycans (Flaibani *et al.*, 1989).

Polysaccharide engineering

The genetic basis for the biosynthesis of bacterial cellulose has been studied at the Institute of Biotechnology for many years. This work is led by Svein Valla, and is now carried out at UNIGEN. A cellulose-negative mutant of *Acetobacter xylinum* produces a water-soluble polysaccharide called xylinan (Fig. 6) with interesting physical properties. We have chosen this system as our model for polysaccharide engineering, a new and rapidly growing field which by means of modern genetic methods

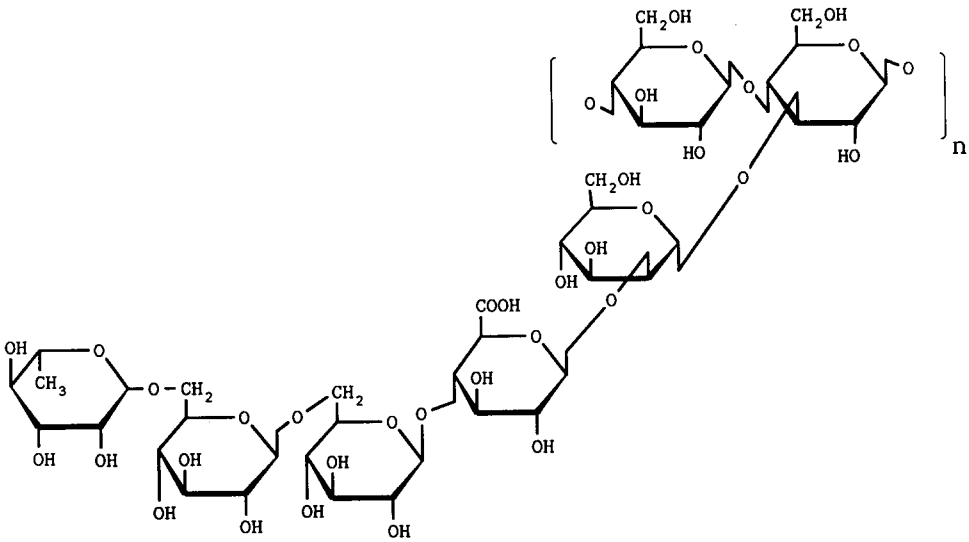


Fig. 6. Tentative structure of the polysaccharide xylinan.

aims at complete control over polysaccharide biosynthesis and the production of new polysaccharides with predetermined properties. The genetic studies of xylinan are performed by Dag H. Coucheron and Tore-Geir Iversen. It now seems that the xylinan system has much in common with xanthan, which has been successfully 'engineered' by the Synergen group in the USA.

Production of viscous polysaccharides and the optimization of fermentation conditions with respect to physical properties are relatively new challenges to chemical engineers. David Levine and Svein Ramstad use xylinan as a model product for this work. The work utilizes the new pilot plant for bioprocesses which is partly owned by and located within the university.

Biopolymer engineering also implies theoretical calculations of physical properties based on the primary structure. Such work on polysaccharides has been initiated in Trondheim by Bjørn T. Stokke in cooperation with David Brant (University of California, Irvine, USA). Further work will include the use of the Cray supercomputer which is located in Trondheim. In addition to the prediction of the equilibrium properties, Arnljot Elgsæter has initiated theoretical studies of biopolymer dynamics for predicting rheological behaviour. As elaborated below, these studies are matched with experimental work using specially designed equipment.

Biophysical instrumentation for polysaccharide solutions and gels

In the late 1970s, Arnljot Elgsæter at the Institute of Physics built up a research group which focused its work on membrane and macromolecule biophysics. The development and construction of new biophysical instrumentation, including computer-assisted systems for instrument control and data acquisition, has been, and still is, a major activity within the group. Some of the major instruments which are available in the group are as follows:

- Cartesian diver low-shear viscometer (highly dilute polymer solutions).
- Gravitational-pendulum viscoelastometer (polymer solutions and soft gels).
- Multiple-lumped resonator (MLR) viscoelastometer (dilute polymer solutions, 100–8000 Hz).
- Torsional-pendulum viscoelastometer (gels, 1–400 bar, 0–100°C).
- Optical rotational dispersion (ORD) spectrometer (1–1000 bar, 0–100°C).

In the early 1980s, emphasis was placed on the development of preparation methods for electron microscopy, e.g. a freeze-fracture device with facilities for rotary shadowing of macromolecules. More recently, the emphasis has been on rheological methods, including instrumentation for studies of biopolymers at high pressures and temperatures.

The development of such instrumentation is also linked to theoretical studies of the conformation and dynamics of biomolecules in solutions and gels. Currently, Arnljot Elgsæter and colleagues Arne Mikkelsen, Bjørn T. Stokke and Kenneth Knudsen in addition to PhD students are initiating detailed theoretical studies on the dynamic behaviour of macromolecules and consequent prediction of bulk properties of

macromolecular solutions and gels. For experimental control of the theoretical work a new generation of instruments is being developed. These include a new birefringence apparatus for studies of elongational flow (at ultra-high strain) and a MLR viscoelastometer operating at high frequencies.

The Norwegian biopolymer laboratory (NOBIPOL)

In the mid-1980s, closer cooperation between the research groups led by Olav Smidsrød and Arnljot Elgsæter gave rise to the idea of forming a joint research group in fields of mutual interest, with the possibility of synergistic effects. At the same time, the University of Trondheim was encouraging the formation of such groups. In 1988, the development of a Biotechnological Centre at the University of Trondheim was formally launched to facilitate cooperation between different groups related to biotechnology, and NOBIPOL was the first group to be formed according to the idea and purpose of the centre.

Directed by Olav Smidsrød and Arnljot Elgsæter, NOBIPOL initiates and coordinates research programmes related to biopolymers. The basis for NOBIPOL is the scientific staff at the two institutes involved as well as temporarily employed research scientists and PhD students. The major activity within NOBIPOL is a NOK 6 mill. national research programme named 'Industrial application of biopolymers'. The programme has essentially three parts, namely alginate and chitosan, biopolymers for enhanced oil recovery, and biopolymer engineering.

THE FUTURE

With the increasing interest in polysaccharide engineering as well as new and exciting applications of polysaccharides, e.g. in the biomedical field and in immobilization materials, research on polysaccharides is expected to expand. We will probably see a new range of polysaccharides with tailor-made properties, due to genetic engineering and advanced fermentation and purification methods. We also expect to be able to predict physical properties based on primary structure. This is a development in which polysaccharide chemists and physicists in Trondheim will take part, and which will involve extended cooperation with related research groups both nationally and internationally.

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