Cah. Biol. Mar. (2001) 42 : 159-167

Organic derivatives of algal polysaccharides

Betty MATSUHIRO
Departamento de Ciencias Químicas, Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 40, Correo 33, Santiago, Chile.
Fax: 56-02-6812108. E.mail: bmatsuhi@lauca.usach.cl

Abstract: A survey of organic derivatives of commercially important red seaweed polysaccharides is presented. The most important applications of soluble polysaccharides extracted from seaweeds utilize their ability to modify the properties of aqueous solutions. The presence of natural substituents and synthetic derivatives have broadened the area of applicability of phycocolloids. The ability of polysaccharides, such as agarose to form hydrophilic supports of well defined dimensions has been exploited for the purification of biological molecules. By appropiate modification of the polysaccharide matrices, derivatives useful in the field of medicine, biology and, biotechnology have been developed. Selective chemical modifications may facilitate the preparation of derivatives and conjugates with novel applications. Treatment of polysaccharides with bromine and periodate may introduce ketone or aldehyde functions, respectively. Neutral polysaccharides, such as agarose and xylan, were oxidized with aqueous bromine and then coupled with amines to give conjugates with potential biological applications. On the other hand, formation of Schiff bases afforded derivatives with metal chelating properties. One of the recent seaweed polysaccharide manipulation involves conjugates with protein carriers, which can be artificial immunogen. Alginic acid, from *Lessonia trabeculata* and its homoguluronic enriched fraction by reaction with caprolactam gave amido derivatives which formed covalent bonding with tetanic toxoid protein. Introduction of keto group in alginic acid, followed by selective acetylation gave analogs of *Salmonella typhi* capsular polysaccharide. These analogs can be linked to carrier proteins by reductive amination.

Résumé: Cette revue décrit les principaux dérivés organiques obtenus à partir de polysaccharides d'algues rouges ayant une importance commerciale. Les applications les plus importantes des polysaccharides solubles, extraits des algues, utilisent leur capacité à modifier les propriétés physiques des solutions aqueuses. La disponibilité de substituts naturels et de dérivés synthétiques a élargi le champ d'application des phycocolloides. La capacité de polysaccharides, tels que l'agarose pour former des supports hydrophiles de dimensions bien définies a été exploitée pour la purification de molécules biologiques. En modifiant d'une façon appropriée les dérivés des matrices de polysaccharides, des dérivés utiles en médecine, biologie et biotechnologies, ont été développés.

Des transformations chimiques sélectives pourraient aussi faciliter la préparation de dérivés et de conjugués avec des nouvelles applications. Le traitement des polysaccharides avec du brome et du periodate peut introduire respectivement des fonctions cétone ou aldéhyde. Les polysaccharides neutres, tels que l'agarose et le xylane, ont été oxydés avec du brome aqueux et ont subi une amination pour produire des conjugués ayant des applications biologiques potentielles. D'autre part, la formation de bases de Schiff produit des dérivés avec des propriétés chélatrices de métaux.

Une des manipulations récentes des polysaccharides des algues marines implique des conjugués avec des protéines qui peuvent être des immunogènes artificiels. L'acide alginique de *Lessonia trabeculata* et sa fraction homoguluronique, en réaction avec du caprolactame, produit des dérivés amidiques qui forment des liaisons covalentes avec la protéine toxique du tétanos. L'introduction d'un groupement cétone dans l'acide alginique, suivi d'une acétylation sélective a produit des analogues du polysaccharide capsulaire de S*almonella typhi*. Ces analogues peuvent se lier à des protéines par amination réductive.

Keywords: agar, alginate, carrageenans, xylan, organic derivatives.

Introduction

Polysaccharides are high molecular-weight carbohydrates. They contain repeating structures of monosaccharides glycosidically joined. The majority of natural polysaccharides contain 80 to 100 sugar residues. Only a very few monosaccharides and modified monosaccharides are present in natural polysaccharides. Commonly occuring sugars are D-glucose, D-mannose, D-galactose, D-fructose, D-xylose and L-arabinose. Among the modified sugars there are L-fucose, L-rhamnose, D-galactosamine, D-glucosamine, D-galacturonic acid, D-glucuronic acid, L-guluronic acid, L-iduronic acid, and D-mannuronic acid. The structure and configuration of the constituent sugars of polysaccharides are covered in current textbooks of organic chemistry (McMurry,1992; Wade, 1995; Solomons, 1996; Volhard & Schore, 1999). Rules of carbohydrate nomenclature have been recently reviewed (McNaught, 1997).

The most abundant polysaccharides contain a single type of monosaccharide units. Frequently, a unique mode of linkage is repeated through the chain. An example is cellulose which is formed by condensation of the hydroxyl on C-1 of a D-glucose unit with the hydroxyl of C-4 on the adjoining glucose unit.

When two types of monosaccharides occur in a polysaccharide, they are linked generally in an ordered arrangement. Even when three or more monosaccharides are present in a polysacharide, they are not linked randomly but are combined in a regular arrangement.

The chemical characterization, structure determination, and physico-chemical properties of polysaccharides have been covered in the literature (Aspinall, 1982; Kennedy & White, 1990; White & Kennedy, 1990; Dumitriu et al., 1996; Kajiwara & Miyamoto, 1998).

The polysaccharides in nature, play different functions. They may act as structural components, storage materials, and as protective substances. The matrix polysaccharides are structural polysaccharides characterized by their gel-forming properties.

The majority of seaweeds synthesized matrix polysaccharides which are extractable by hot water, dilute acid, or alkali. The most important applications of these soluble polysaccharides, the phycocolloids, utilize their ability to modify the properties of aqueous solutions. Commercially available polysaccharides obtained from red algae (Rhodophyta) are the three types of carrageenan, designated κ, ι and λ, and agar, from which agarose is isolated by fractionation. Alginic acid is produced by brown algae (Phaeophyta) (Painter, 1983). Phycocolloids are used as functional ingredients in the food, pharmaceutical and cosmetic industries, where by controlling the physico-chemical properties of the aqueous phase, they confer desired properties such as stability, texture, thickness, etc.. The industrial applications of seaweed polysaccharides have been reviewed in several papers (Sandford & Baird, 1983; Yalpani & Sandford, 1987; Renn, 1990; Griffiths & Kennedy, 1990; Armisen, 1995; Bixler, 1996).

The presence of natural substituents and synthetic derivatives have broadened the area of applicability of the polysaccharides.

Much of the chemistry of polysaccharides is the chemistry of alcohols. In native polysacharides, substitution can take place in free primary and secondary alcoholic functions. Commonly, some of the hydroxyl groups, can be etherified or esterified, and the hydroxyl groups on C-4 and C-6 positions may be derivatized by pyruvic acid. Polysaccharides containing uronic acids usually exist in salt forms, but in pectins the carboxyl groups are methylated (Rolin et al., 1998).

Derivatives of polysaccharides can be obtained by chemical or enzymatic modifications of the hydroxyl groups. An important reaction of alcohols is their oxidation to yield carbonyl compounds. Primary alcohols yield aldehydes or carboxyllic acids, and secondary alcohols yield ketones. Oxidation of alcohol functions can be carried out by a large number of chemical reagents. Oxidation can be catalyzed by enzymes. For example, the enzymic oxidation of D-galactose containing polysaccharides by galactose oxidase allow the introduction of reactive C-6 aldehydes functions. The oxidized polysaccharide can be derivatized by reductive amination reaction which allow the incorporation of organic substituents or biological substrates (Yalpani, 1985).

Enzymatic modifications occur in nature, the best example is the conversion of galactose 6-sulfate to 3,6-anhydrogalactose in red seaweeds polysaccharides (Rees, 1961; Wong & Graigie, 1978). Haug & Larsen (1971) have used an enzyme from *Azobacter vinelandii* for the epimerization of D-mannuronic acid residues to L-guluronic acid in alginate sample.

The objective of this overview is to present the natural derivatives of commercially important red seaweed polysacharides, agar and carrageenans, and the synthetic derivatives of the neutral fraction of agar, agarose and alginic acid. Results obtained in the synthesis of organic derivatives of alginic acid and xylan by chemical modifications are also included.

Naturally-occurring derivatives

According to Duckworth & Yaphe (1971a), and Izumi (1972) agar is a family of related polysaccharides having the same backbone structure (alternating 1→3-linked β-D-galactopyranosyl and 1→4-linked α-L-galactopyranosyl

residues), but substituted to a variable degree with charged groups. Agarose, one extreme of structure in agar, is composed of alternating 1→4-linked 3,6-anhydro-α-L-galactopyranosyl and 1→3 linked β-D-galactopyranosyl residues containing no charged groups. The second extreme is a sulfated galactan. Two organic groups are found attached to the agar backbone, methyl ethers and pyruvic ketal.

The most important property of agar is the ability to form reversible gels at very low concentrations, by cooling hot aqueous solutions. The gelling process is reversible. The gel melts on heating and resets on cooling. Guiseley (1970), in a classical work, reported that the gelling temperature of agarose (1,5% in water) increases with increasing methoxyl content. But, when low methoxyl agarose was methylated, a decrease in gelling and melting temperature was found (Guiseley, 1987). The decrease is dependent on the degree of substitution.

The methyl group is attached to the primary alcoholic group on D-galactopyranosyl residues or to secondary alcoholic groups (Brasch et al., 1983). Agars extracted from species of the genus *Gracilaria* contain between 15-30% of 6-O-methylgalactopyranose and those from *Gelidium* contain no more than 5%.

4-O-Methy-D-galactopyranosyl residues were found in agar from *Gelidium amansii* (Araki et al., 1967), and those extracted from *Gracilaria foliifera, G. damaecornis, G. domingensis* and *G. ferox* (Duckworth et al., 1971). It was shown that this residue occured as a single branch-unit, attached to O-6 of the D-galactopyranose units (Craigie & Jurgens, 1989; Karamanos et al., 1989). Methylation occurs also on the secondary hydroxyl group of carbon 2 in the 1,4-linked 3,6-L-anhydrogalactopyranosyl residues (Araki & Hirase,1960; Ji et al., 1985; Stevenson & Furneaux; 1991; Murano et al., 1992).

Dimethylation on O-6 of D-galactopyranosyl and O-2 of 3,6-anhydro-L-galactopyranosyl residues was found in the agar from *Curdiae coriacea* while agars from *C. flabellata* and *Melanthalia abbscissa* showed partial dimethylation (Furneaux et al., 1990). The agar from *Gracilaria chilensis* collected in Chile is partially dimethylated at O-6 of D-galactopyranose units and at O-2 of the 3,6-anhydro-L-galactopyranose units. Partial methylation in the 3,6-anhydro-L-galactose units was mainly found in the neutral, "agarose type" fraction (Matsuhiro & Urzúa, 1994a).

The content of 6-O-methylgalactose in agars from samples of *Gracilaria chilensis* collected at different locations along the northern coast of Chile varies between 3.3 to 27.0%. It is noteworthy that agar samples with a higher content of this methylated sugar also contain a higher amount of sulfate groups. Chemical analysis indicated that the major amount of 6-O-methylgalactose is presented in the neutral fractions (Matsuhiro & Urzúa, 1994b).

Pyruvic acid is attached to the hydroxyls groups on carbons 4 and 6 of the 1,3-linked D-galactopyranosyl residues and its content in agars varies between 0-4% (Hirase, 1957). Young et al. (1971) analysed a variety of agars and found that agars with high sulphate values would have low pyruvate levels. Combined studies by enzymic hydrolysis and nuclear magnetic resonance spectroscopy (NMR) indicated that the majority of pyruvate groups were present in agarose type fragments (Duckworth & Yaphe, 1971b; Bhattacharjee & Yaphe, 1979; Lahaye et al., 1989).

Methoxyl and pyruvate groups are regarded as the common organic substituents of agars but are less common in carrageenans. Carrageenans are produced by members of the Gigartinaceae and Phyllophoraceae. In carrageenans, the 4-linked residue is in the D-configuration and is typically more sulfated than agars. The structure of different types of carrageenans have been extensively reviewed (Painter, 1983; Usov, 1992, 1998).

Minor amounts of methylated galactoses have been detected in carrageenans from some species of Gigartinaceae and Phyllophoraceae (Whyte et al., 1984; Usov & Shaskov, 1985; Ibañez & Matsuhiro, 1986; Ayal & Matsuhiro, 1986). Recently, Chiovitti et al. (1996) informed that *Rhabdonia coccine* and *R. verticillata* of the family Solieriaceae, produced a ι-carrageenan type polysaccharide, partially methylated at O-6 of the 3-linked galactose residue (31 mol% and 17 mol%, respectively). The polysaccharide from *R. verticillata* also contained pyruvic acid and the unusual sugar, 3-O-methylgalactose.

Pyruvate residues were previously detected in λ-carrageenan type polysaccharides from *Petrocelis middendorfii*, *Gigartina* spp., *Kallymenia westii* (DiNino et al, 1979; Hirase & Watanabe, 1972; Stevenson & Furneaux, 1991). Chiovitti et al. (1998), reported that the carrageenan from *Sarconema filiforme* is a hybrid or mixture of α- carrageenan (→3-β-D-galactopyranosyl-1→4)-3,6-anhydro-α-D-galactopyranosyl-1), ι-carrageenan and pyruvated α-carrageenan, with low levels of methylation.

Synthetic derivatives

By etherification or esterification simple derivatives of agarose, such as methyl, hydroxyethyl, hydroxypropyl, allyl and acetyl derivatives may be obtained. Among the most important commercial organic derivatives of agarose are the hydroxyethylated products. Gels of these derivatives have markedly lower melting points than those of agarose, which permitted its use in molecular biology laboratories. It is used for the electrophoretic separation of DNA fragments, since after the electrophoresis, the band of interest can be cut out, the gel melted and the DNA separated from the agarose gel (Guisely, 1987).

Another interesting derivative of agarose is the allyl glyceryl ether, obtained by reaction of agarose with allyl glycidyl ether in basic conditions, according to the scheme in Fig. 1. It is used for the preparation of polyacrylamide gels on plastic films for electrophoretic separations.

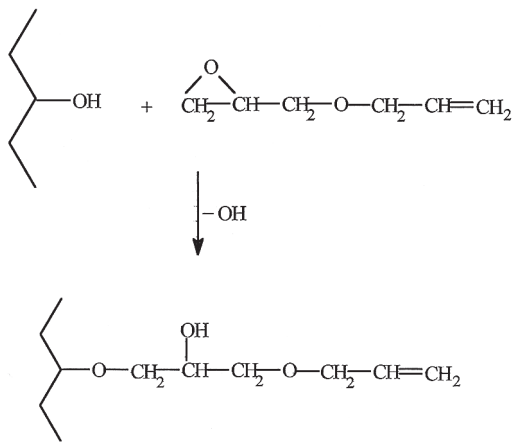

Figure 1. Allyl glyceryl ether of agarose.
Figure 1. Allyl glyceryl ether de l'agarose.

The ability of polysaccharides to form hydrophilic supports of well defined dimensions has been exploited for the purification of biological molecules. Separation of biopolymers by size-exclusion chromatography on agarose is a classical example. By appropiate modification of agarose, matrices useful in the field of medicine, biology and, biotechnology have been developed. For example, in biochemicals products catalogues a large list of organic derivatives of agarose for affinity chromatography is found. A variety of reagents and methods for the activation of agarose for affinity chromatography are used. Usually, agarose is first derivatized by reaction with cyanogen bromide, carbodiimide, carbonylimidazol, epoxy, N-hydroxysuccinimide ester, iodoacetyl or hydrazide. The activation of agarose with carbonylimidazol and coupling of a protein is shown in Fig. 2.

The reaction with a free amino group of the protein creates a stable chemical link (covalent linkage) between the ligand and the agarose matrix. In Fig. 3 commercially availble organic derivatives of agarose used in the coupling of biological important molecules are presented.

The hydroxyl groups on the surface of agarose, by reaction with tresyl chloride in basic medium, gave a sulfonated derivative that can be used for primary amine or thiols coupling (a). By reaction of agarose with diglycidyl ether of 1,4-butan-diol, an epoxide is formed in basic conditions. Soybean trypsin inhibitor was immobilized on

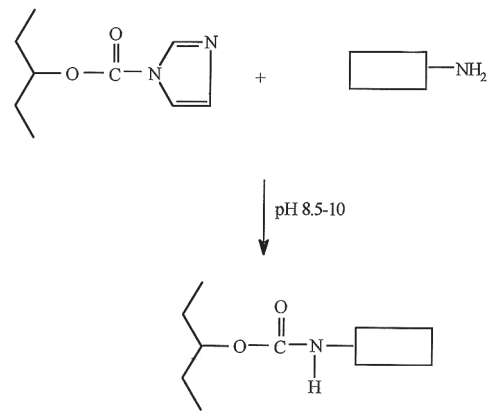

Figure 2. Activation of agarose and coupling to protein.
Figure 2. Activation de l'agarose et couplage à une protéine.

the epoxy derivative of agarose at pH 7.8 (b). p-Chloromercuribenzoate immobilized on a cross-linked 6% beaded agarose through an ethylediamine spacer is sold for coupling protein or other biomolecules through sulfhydryl groups (c).

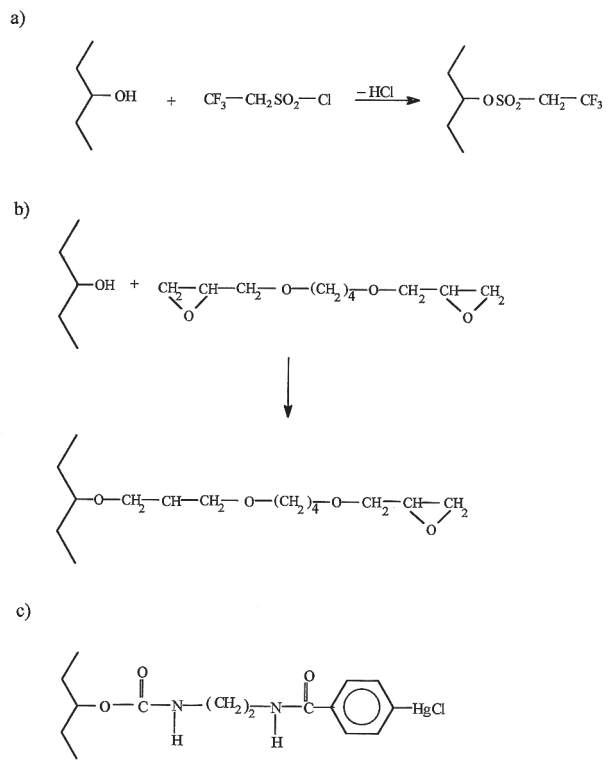

Figure 3. Organic derivatives of agarose.
Figure 3. Dérivés organiques de l'agarose.

In these applications, little attention was often paid to elucidate the chemical structure of the reaction products. Selective chemical modifications may facilitate the preparation of derivatives and conjugates with novel applications. Treatment of polysaccharides with bromine and periodate may introduce ketone or aldehyde functions, respectively. Neutral seaweed polysaccharides, such as agarose and xylan were oxidized with aqueous bromine and then coupled with amines to give conjugates with potential biological applications.

The main water-soluble polysaccharides extracted from members of the Palmariales and Nemaliales are neutral xylans. Xylans represent an interesting source of raw material for the preparation of modified polysaccharides with potential applications. Having no primary alcoholic groups, they should form few side products on oxidation. Treatment of the xylan from *Palmaria decipiens* with bromine afforded a water soluble derivative with 66% of yield. By ^{13}C NMR spectroscopy and formation of a Schiff base, the presence of the keto group was confirmed (Fig. 4). The oxidation in position C-2 was verified by reduction and total hydrolysis of the modified polysaccharide, and by gas-liquid chromatography analysis of the alditol acetates. These results are in accordance with those obtained by Salomonsson & Theander (1992) on the selective bromine oxidation of starch on C-2. When the reaction with the amine was conducted in the presence of sodium cyanoborohydride, a reducting agent, a secondary amine group was attached to the polysaccharide. The same reaction with bovine serum albumin (BSA) afforded a polysaccharide-protein conjugate (Jerez et al., 1997) (Fig. 5).

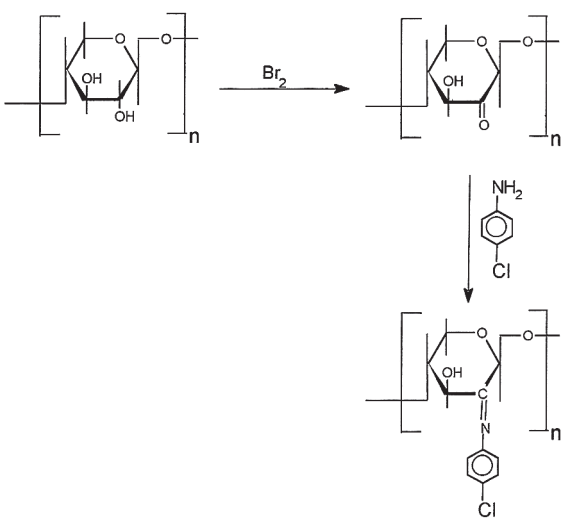

Figure 4. Oxidation of xylan and formation of a Schiff base.
Figure 4. Oxydation du xylane et formation d'une base de Schiff.

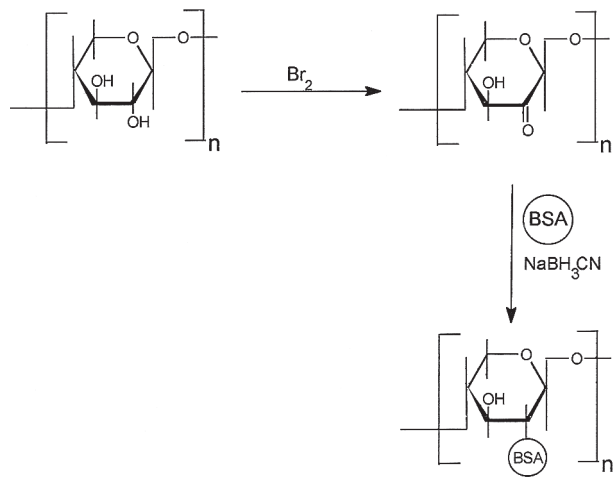

Figure 5. Conjugation of oxidized xylan to BSA by reductive amination.
Figure 5. Conjugaison du xylane oxydé à la BSA par amination réductrice.

On the other hand, oxidation of the xylan with sodium periodate allowed the introduction of aldehyde functions. The modified xylan was treated with p-chloroaniline to give ligands for the coordination of Cu(II). The chemical modifications of polysaccharides was easily followed by the second-derivative of the FT-IR spectra. Coordination complexes of transition metals with polysaccharide derived ligands have potential applications as catalysts, in asymmetric synthesis (Barroso et al., 1997).

Larm et al. (1979) obtained an heparin analog, an anticoagulant drug, by bromine oxidation of a partially reduced alginic acid and subsequent reductive amination (Fig. 6).

Alginic acid is a copolymer of D-mannuronic and L-guluronic acids arranged in homopolymeric and heteropolymeric blocks. The capacity of alginates to form gels with calcium salts is an important property (Painter, 1983).

Esterification of hydroxyl groups is one of the most important chemical procedures for changing properties of polysaccharides. Treatment of alginic acid with acetic anhydride and catalytic amounts of perchloric acid afforded per-o-acetylated products that not gel in the presence of calcium ions. The maximum value of viscosity for the aqueous solution of ammonium acetylated alginic acid was otained between a degree of acetylation of 0.5-0.8 (Schweiger, 1962). When the reaction of acetylation is carried on calcium alginate or alginic acid gels in heterogeneous media with acetic anhydride and pyridine, the substitution takes place on the mannurosyl residues. The derivative showed exceptional swelling properties (Skjäk-Braek et al., 1989).

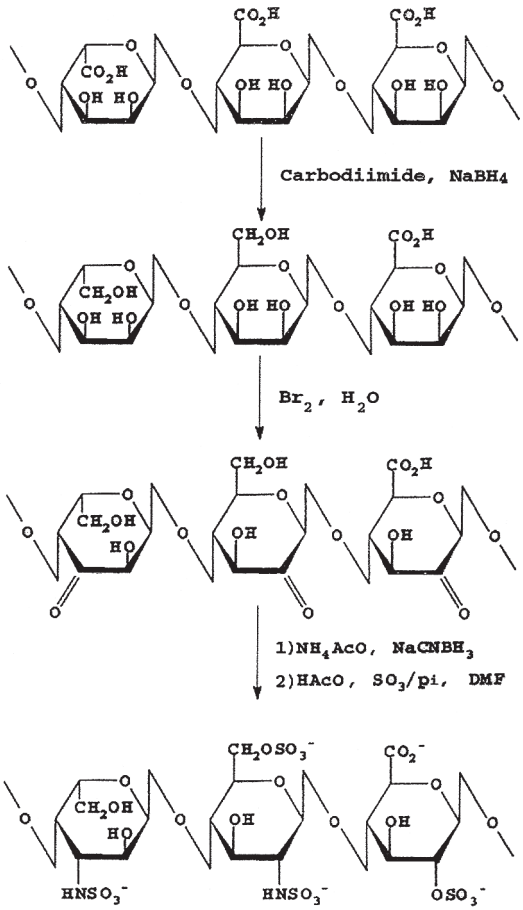

Figure 6. Chemical modification of alginic acid. Obtention of a analog of heparin.
Figure 6. Modification chimique. Obtention d'un analogue de l'héparine.

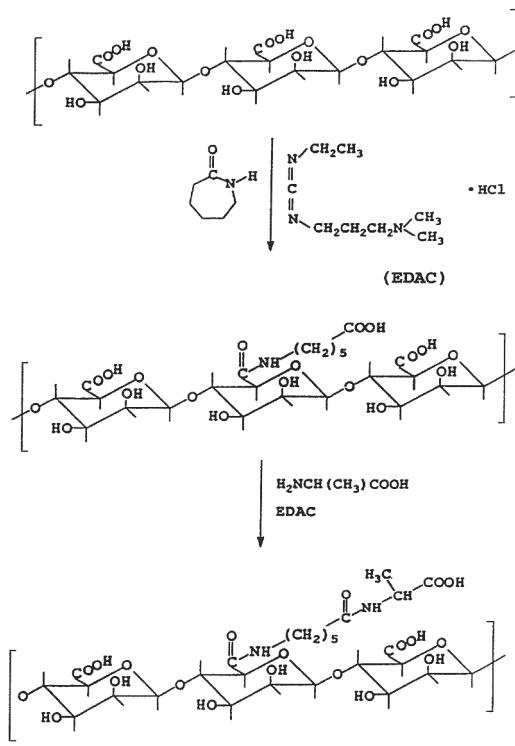

Figure 7. Reaction of alginic acid extracted from *Lessonia trabeculata* with caprolactam and conjugation with an aminoacid.
Figure 7. Réaction de l'acide alginique extrait de *Lessonia trabeculata* avec du caprolactame et conjugaison avec un acide aminé.

The propylene glycol ester of alginic acid is an excellent thickener and stabilizer. The degree of esterification can also vary. It is used to suspend the fruit pulp in non-carbonated fruit drinks and in many beers to stabilize the foam (Sandford & Baird, 1983).

One of the recent seaweed polysaccharide manipulation involves conjugates with protein carriers, which can be artificial immunogen. *Lessonia trabeculata* Villouta & Santelices, of the order Laminariales, forms extensive kelp beds in Chilean subtidal environments. Differences in M/G and block composition in the alginic acids from populations collected at different ecological locations were found (Matsuhiro & Zambrano, 1989; Venegas et al., 1993).

A purified fraction of alginic acid from *Lessonia trabeculata* was activated with carbodiimide and a spacer arm was introduced by reaction with caprolactam (Fig. 7). The new terminal carboxylic function was used to create a covalent link with the amino groups of basic aminoacid residues of BSA. In this way a seaweed polysaccharide-protein conjugate with antigenic properties was synthesized (Lillo et al., 1996). The same alginic acid derivative gave with tetanus toxoid in the presence of carbodiimide a polysaccharide-protein conjugate in good yield (Lillo et al., 1999). These conjugates constitute excellent models for antigenic conjugates that may be used as vaccines against infectious deseases.

In humans, *Salmonella typhi* is the causative agent of typhoid fever which continues to be a widespread and serious disease in the developing world. It produces a capsular polysaccharide known as Vi polysaccharide (for virulence), a linear polymer of N-acetyl-2-amino-2-deoxy-D-galacturonic acid 90% acetylated on carbon 3. Vaccines composed of Vi polysaccharide confer immunity in adults and children older than five years of age. The efficacy of polysaccharide-based vaccines may be improved by covalent linking of oligo- or polysaccharides to protein carriers (Robbins & Schneerson, 1990, Szu et al., 1987, 1989, 1991).

Partial hydrolysis of alginic acid with low M/G value, extracted from *Lessonia trabeculata* afforded a fraction enriched in polyguluronic sequences. The structure of this

homoplymer is almost the mirror image of the poly-D-galucturonic acid, the backbone structure of Vi polysaccharide. Oxidation with bromine allows the introduction of a keto group, which by reductive amination, followed by per-O-acetylation gives an analog of *Salmonella typhi* capsular polysaccharide (Chandía & Matsuhiro, unpublished). If the reductive amination takes place with the amino group of a protein, a conjugate may be obtained. The analog can be linked to carrier proteins by two sequential amidations in the presence of carbodiimides. The conjugates may lead to potential uses in the diagnosis and prevention of typhoid fever.

Conclusions

Phycocolloids are important members of industrial water soluble biopolymers. They are biodegradable, non-toxic materials obtained from renewable resources. Chemical and enzymatic modifications would improve the physico-chemical properties of seaweed polysaccharides and provide new uses. Polysaccharides organic derivatives can be sources of high-value products. With a better understanding of the relationship between structure of polysaccharides and their functional activities, compounds with specific properties may be obtained by chemical modifications. New modified polysaccharides are likely to be low-volume, high cost, fine chemicals or biological active materials.

Acknowledgements

The financial support of Dirección de Investigaciones of Universidad de Santiago de Chile and FONDECYT, Grant 1980828 is gratefully acknowledged.

References

Araki C., Arai K. & Hirase S. 1967. Studies on the chemical constitution of agar-agar. XXIII. Isolation of D-xylose, 6-O-methyl-D-galactose, 4-O-methyl-L-galactose and O-methylpentose. *Bulletin of the Chemical Society of Japan,* **40**: 959-962.

Araki C. & Hirase S. 1960. Studies on the chemical constitution of agar-agar. XXI. Re-investigation of methylated agarose of *Gelidium amansii*. *Bulletin of the Chemical Society Japan*, **29**: 339-345.

Armisen R. 1995. World-wide use and importance of *Gracilaria*. *Journal of Applied Phycology,* **7**: 231-243.

Aspinall G.O. 1982. *The Polysaccharides*. Vol. **1**. Academic Press: New York. 340 pp.

Ayal H.A. & Matsuhiro B. 1986. Carrageenans from tetrasporic and cystocarpic *Chondrus canaliculatus*. *Phytochemistry*, **25**: 1895-1897.

Barroso N.P., Costamagna J., Matsuhiro B. & Villagrán M. 1997. El xilano de *Palmaria decipiens*: modificación química y formación de un complejo de Cu(II). *Boletín de la Sociedad Chilena de Química,* **42**: 301-306.

Bhattacharjee S. & Yaphe W. 1979. Enzymic and N.M.R. spectroscopic analyses of agar-type polysaccharides. In: *Marine Algae in Pharmaceutical Science* (H.A. Hoppe, T. Levring & Y. Tanaka eds*)*, pp. 645-654. Walter de Gruyter: New York.

Bixler H.J. 1996. Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia,* **326/327**: 35-57.

Brasch D.J., Chuah C.T. & Melton L.D. 1983. The agar-type polysaccharide from the red alga *Gracilaria secundata*. *Carbohydrate Research,* **115**: 191-198.

Chiovitti A., Bacic A., Craik D.J., Kraft G.T., Liao M.-L, Falshaw R. & Furneaux R.H. 1998. A pyruvated carrageenan from Australian specimens of the red alga *Sarconema filiforme*. *Carbohydrate Research,* **310**: 77-83.

Chiovitti A., Liao M.-L., Kraft G.T., Munro S.L.A., Craik D.J. & Bacic A. 1996. Cell wall polysaccharides from australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): highly methylated carrageenans from the genus *Rhabdonia*. *Botanica Marina*, **39**: 47-59.

Craigie J. S. & Jurgens A. 1989. Structure of agars from *Gracilaria tikvahiae* Rhodophyta: location of 4-O-methyl-L-galactose and sulphate. *Carbohydrate Polymers*. **11**: 265-278.

DiNinno V., McCandless E. L. & Bell R.A. 1979. Pyruvic acid derivative of a carrageenan from marine red alga (*Petrocelis* species). *Carbohydrate Research,* **71**: C1-C4.

Duckworth M., Hong K.C. & Yaphe W. 1971. The agar polysaccharide of *Gracilaria* species. *Carbohydrate Research,* **18**: 1-9.

Duckworth M. & Yaphe W. 1971a. The structure of agar. Part 1. Fractionation of a complex mixture of polysaccharides. *Carbohydrate Research,* **16**: 189-197.

Duckworth M. & Yaphe W. 1971b. The structure of agar. Part II. The use of bacterial agarase to elucidate structural features of the charged polysaccharides in agar. *Carbohydrate Research*, **16**: 435-445.

Dumitriu S., Vidal, P.F. & Chornet, E. 1996. Hydrogels based on polysaccharides. In: *Polysaccharides in medicinal applications* (S. Dumitriu ed), pp. 125-241. Marcel Dekker: New York.

Furneaux R.H., Miller I. J. & Stevenson T. T. 1990. Agaroids from New Zealand members of the Gracilariaceae. A novel dimethylated agar. *Hydrobiologia,* **204/205**: 615-620.

Griffiths A.J. & Kennedy J.F. 1990. Biotechnology of polysaccharides. In*: Carbohydrate chemistry* (J.F. Kennedy ed), pp. 597-635. Clarendon Press: Oxford.

Guiseley K.B. 1970. The relationship between methoxyl content and gelling temperature of agarose. *Carbohydrate Research,* **13**: 247-256.

Guiseley K.B. 1987. Natural and synthetic derivatives of agarose and their use in biochemical separations. In: *Industrial Polysaccharides: genetic engineering, structure/property relations and applications* (M. Yalpani ed), pp. 139-147. Elsevier: Amsterdam.

Haug A. & Larsen B. 1971. Biosynthesis of alginate. Part II. Polymannuronic acid C-5-epimerase from *Azotobacter vinelandii* (Lipman). *Carbohydrate Research,* **17**: 297-308.

Hirase S. 1957. Studies on the chemical constitution of agar-agar. XIX. Pyruvic acid as a constituent of agar-agar (Part 3). Structure of the pyruvic acid-linking disaccharide derivative isolated from methanolysis products of agar. *Bulletin of the Chemical Society of Japan,* **30**: 75-79.

Hirase S. & Watanabe K. 1972. The presence of pyruvate residues in λ-carrageenan and a similar polysaccharide. *Bulletin of the Institute for Chemical Research, Kyoto University* **50**: 332-336.

Ibañez C. M. & Matsuhiro B. 1986. Structural studies on the soluble polysaccharide from *Iridaea membranacea*. *Carbohydrate Research,* **146**: 327-354.

Izumi K. 1972. Chemical heterogeneity of the agar from *Gracilaria verrucosa*. *Journal of Biochemistry,* **72**: 135-140.

Jerez J., Matsuhiro B. & Urzúa C.C. 1997. Chemical modifications of the xylan from *Palmaria decipiens*. *Carbohydrate Polymers,* **32**: 155-159.

Jerez J., Matsuhiro B., Urzúa C.C. & Zúñiga E.A. 1999. Modificación química de ácido algínico y su conjugación a toxoide tetánico. *Boletín Sociedad Chilena Química,* **44**: 227-231.

Ji M., Lahaye M. & Yaphe W. 1983. Structure of agar from *Gracilaria* spp. (Rhodophyta) collected in the People's Republic of China. *Botanica Marina,* **28**: 521-528.

Kajiwara K. & Miyamoto T. 1998. Progress in structural characterization of functional polysaccharides. In: *Polysaccharides* (S. Dumitriu ed), pp. 1-55. Marcel Dekker: New York.

Karamanos Y., Ondarza M., Bellanger F., Christiaen D. & Moreau S. 1989. The linkage of 4-O-methyl-L-galactopyranose in the agar polymers from *Gracilaria verrucosa*. *Carbohydrate Research,* **187**: 93-101.

Kennedy J.F. & White C.A. 1990. Classification and description of monosaccharides, oligosaccharides, and polysaccharides. In: *Carbohydrate Chemistry* (J.F.Kennedy ed), pp. 3-41. Clarendon Press: Oxford.

Lahaye M., Revol J.F., Rochas C., McLachlan J. & Yaphe W. 1988. The chemical structure of *Gracilaria crassissima* (P. & H. Crouan in Schramm et Mazé) P. et H. Crouan in Schramm & Mazé and *G. tikvahiae* McLachlan (Gigartinales, Rhodophyta) cell-wall polysaccharides. *Botanica Marina,* **31**: 491-501.

Lahaye M. & Yaphe W. 1988. Effects of seasons on the chemical structure and gel strength of *Gracilaria pseudoverrucosa* agar (Gracilariaceae, Rhodophyta). *Carbohydrate Polymers,* **8**: 285-301.

Lahaye M., Yaphe W., Phan Viet M.T. P. & Rochas C. 1989. ^{13}C-N.M.R. spectroscopic investigation of methylated and charged agarose oligosaccharides and polysaccharides. *Carbohydrate Research,* **190**: 249-265.

Larm O., Larsson K. & Scholander E. 1979. The preparation of a heparin analogue from alginic acid. *Carbohydrate Research*, **73**: 332-336.

Lillo L.E., Matsuhiro B. & Zúñiga E.A. 1996. Modificación química de polisacáridos acídicos: conjugación y propiedades inmunogénicas. *Actas III Simposio Internacional "Química de productos naturales y sus aplicaciones*", Punta de Tralca: 256-257.

Matsuhiro B. & Urzúa C.C. 1994a. 2-O-metil-3,6-anhidro-galactosa en agar de *Gracilaria chilensis*. *Boletín de la Sociedad Chilena de Química,* **39**: 179-182.

Matsuhiro B. & Urzúa C.C. 1994b. Calidad de agar de *Gracilaria chilensis*. *Revista Investigación Científica y Tecnológica, Serie Ciencias del Mar* (Univ. Arturo Prat, Chile), **93**: 71-76.

Matsuhiro B. & Zambrano D. 1989. La relación ácido manurónico a ácido gulurónico en el ácido algínico de *Lessonia trabeculata*. *Boletín de la Sociedad Chilena de Química,* **34**: 21-25.

McMurry J. 1992. Organic Chemistry, pp. 915-956. Brooks/Cole: Belmont.

McNaught A.D. 1997. Nomenclature of carbohydrates. *Carbohydrate Research,* **297**: 1-92.

Miller I.J. & Furneaux R.H. 1987. The chemical substitution of the agar-type polysaccharide from *Gracilaria secundata* f. *pseudoflagellifera* (Rhodophyta). *Hydrobiologia,* **151/152**: 523-529.

Murano E., Toffani R., Zanetti F., Knutsen S.H., Paoletti S. & Rizzo R. 1992. Chemical and macromolecular characterisation of agar polymers from *Gracilaria dura* (C.Agardh) J. Agardh (Gracilariaceae, Rhodophyta). *Carbohydrate Polymers,* **18**: 171-178.

Painter T.J. 1982. Algal polysaccharides. In: *The Polysaccharides* Vol.**1** (G.O.Aspinall ed), pp. 196-285. Academic Press: New York.

Renn D. 1990. Seaweeds and biotechnology-inseparable companions. *Hydrobiologia,* **204/205**: 7-13.

Rees D.A. 1961. Enzymatic synthesis of the 3,6-anhydro-L-galactose within porphyran from L-galactose 6-sulphate units. *Biochemical Journal,* **81**: 347-352.

Robbins J.B. & Schneerson R. 1990. Polysaccharide-protein conjugates: a new generation of vaccines. *Journal of Infection Diseases,* **161**: 821-832.

Rolin C., Nielsen B.U. & Glahn O-E. 1998. Pectins. In: *Polysaccharides* (S., Dumitriu ed), pp. 377-431. Marcel Dekker: New York.

Salomonsson A-C.B. & Theander O. 1992. Coupling of 1-aminododecan to starch by bromine oxidation and reductive amination. *Starch,* **44**: 260-263.

Sanford P. A. & Baird J. 1983. Industrial utilization of polysaccharides. In: *The polysaccharides* (G.O. Aspinall ed), Vol. **2**, pp. 411-490. Academic Press: New York.

Skjäk-Braek G., Zanetti F. & Paoletti S. 1989. Acetylated alginates: the impact of acetyl groups on the functional properties of alginates. In: *Biomedical and biotechnological advances in industrial polysaccharides* (V. Crescenzi, I.C.M. Dea, S. Paoletti, S.S. Stivala & I.W. Sutherlan eds), pp. 385-398. Gordon and Breach Science Publishers: New York.

Solomons T.W.G. 1996. *Organic Chemistry*. 6th ed., pp. 1046-1092. Wiley: New York.

Stevenson T.T. & Furneaux R. H. 1991. Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydrate Research,* **210**: 277-298.

Schweiger R. G. 1962. Acetylation of alginic acid. Preparation and viscosities of algin acetates. *Journal of Organic Chemistry*, **27**: 1786-1789.

Szu S. C., Li X., Schneerson R., Vickers J.H., Bryla D. & Robbins J.B. 1989. Comparative immunogenicities of Vi polysaccharide-protein conjugates composed of cholera toxin or its B subunit as a carrier bound to high or lower-molecular weigth Vi. *Infection and Immunity,* **57**: 3823-3827.

Szu S.C., Stone A.L. & Robbins J.B. 1991. Relation between structure and immunologic properties of the Vi capsular polysaccharide. *Infection and Immunity,* **59**: 4555-4561.

Szu S. C., Stone A. L., Robbins J. D., Schneerson R. & Robbins J. B. 1987. Vi capsular antigen polysaccharide-protein conjugates for prevention of thyphoid fever. Preparation, characterization, and immunogenicity in laboratory animals. *Journal of Experimental Medicine,* **166**: 1510-1524.

Usov A.I. 1992. Sulfated polysaccharides of the red seaweeds. *Food Hydrocolloids,*. **6**: 9-23.

Usov A.I. 1998. Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids,* **12**: 301-308.

Usov A.I. & Shashkov A.S. 1985. Polysaccharides of algae. XXXIV:detection of iota-carrageenan in *Phyllophora brodiaei* (turn.) J. Ag. (Rhodophyta) using ^{13}C-NMR spectroscopy. *Botanica Marina,* **38**: 367-373.

Venegas M., Matsuhiro B. & Edding M.E. 1993. Alginate composition of *Lessonia trabeculata* (Phaeophyta: Laminariales) growing in exposed and sheltered habitats. *Botanica Marina,* **36**: 47-51.

Vollhardt K.P.C. & Schore N.E. 1999. *Organic Chemistry*, 3rd ed, pp. 1068-1115. Freeman: New York.

Wade L.G. 1995. *Organic Chemistry*, 3rd ed, pp. 1103-1163. Prentice Hall: Upper Saddle River.

Whyte J. N. C., Forteman R. E. & DeWreede R. E. 1984. Phycocolloid screening of British Columbia red algae. *Hydrobiologia,* **115/117**: 537-541.

White C.A. & Kennedy J.F. 1990. Identification and structural analysis of monomeric and polymeric carbohydrates. In: *Carbohydrate Chemistry* (J.F. Kennedy ed), pp. 42-72. Clarendon Press: Oxford.

Wong K.F. & Craigie J.S. 1978. Sulfohydrolase activity and carrageenan biosynthesis in *Chondrus crispus* (Rhodophyceae). *Plant Physiology*, **61**: 663-666.

Yalpani M. 1985. A survey of recent advances in selective chemical and enzymic polysaccharide modifications. *Tetrahedron*, **41**: 2957-3020.

Yalpani M. & Sandford P.A. 1987. Commercial polysaccharides: recent trends and developments. In: *Industrial polysaccharides* (M. Yalpani ed), pp. 311-336. Elsevier: Amsterdam.

Young K., Duckworth M. & Yaphe W. 1971. The structure of agar. Part III. Pyruvic acid, a common feature of agars from different agarophytes. *Carbohydrate Research*, **16**: 446-448.