Supramolecular Biopolymers II

Polysaccharides

Chapter 2: Polysaccharides

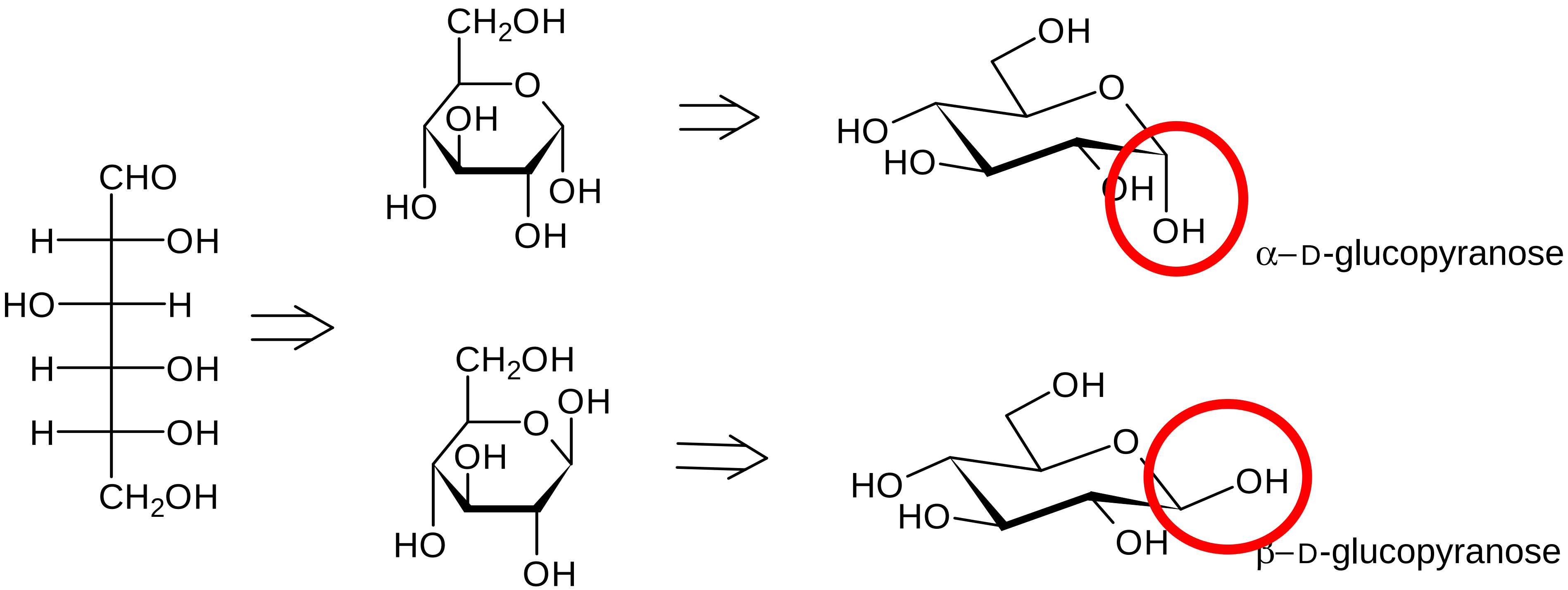
Polysaccharides are ubiquitous biopolymers built up from monosaccharides. They belong to the carbohydrates (sugars). 99% are located in plants. World sugar production: 10^8 tons; world oil production: 40 x 10^8 tons; world cellulose production 100 x 10^8 tons.

Very often, polysaccharides are not pure. They are associated with other polysaccharides, polyphenolics, or proteins, either by covalent or by non-covalent bonds.

Polysaccharides

2.1 **Overview: Monosaccharides and Nomenclature**

- Principles of monosaccharide structures (hexopyranoses only; for derivation of ring structures, see figure):



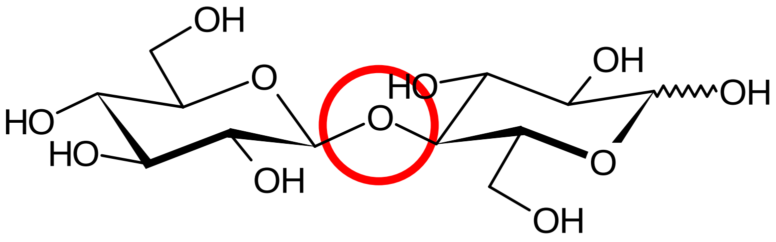
Fischer formula

Haworth formula

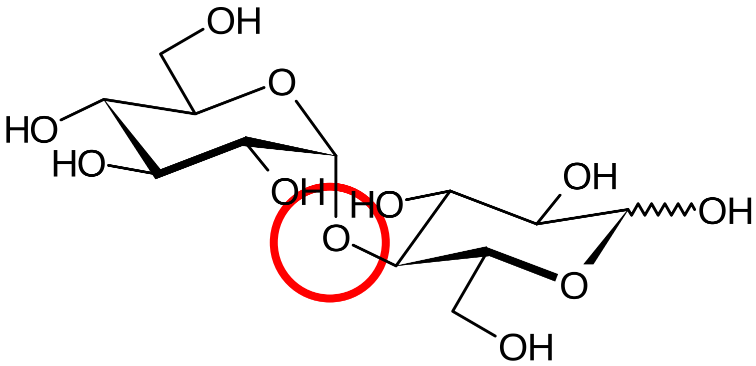
$^4C_1$ chair conformation

Polysaccharides

- The glycosidic bond



β-D-4-glucopyranosyl glucose
β-D-Glcp-(1,4)-Glcp
cellobiose



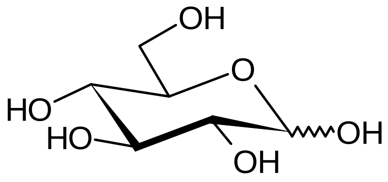
α-D-4-glucopyranosyl glucose
α-D-Glcp-(1,4)-Glcp
maltobiose

Polysaccharides contain frequently small amounts of sugar derivatives, in particular:

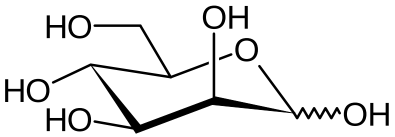
Esters of phosphoric acid (phosphates)
Sulphuric acid (sulfates)
acetic acid (acetates)

α and β bonds lead to fundamentally different secondary structures

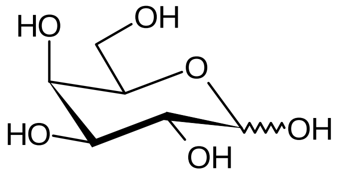
Monosaccharide structures found most commonly in polysaccharides



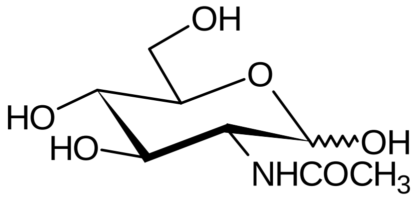
D-glucose
Glc



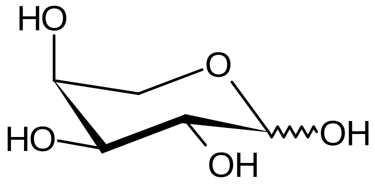
D-mannose
Man



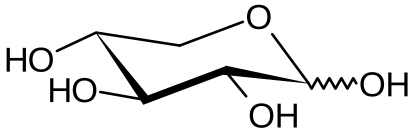
D-galactose
Gal



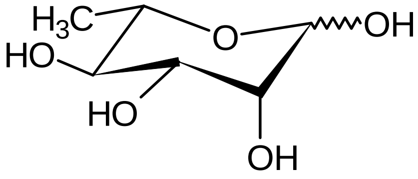
2-acetamido-2-deoxy-D-glucose
GlcNAc



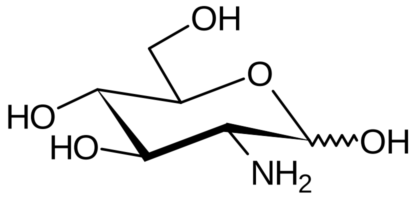
L-arabinose
Ara



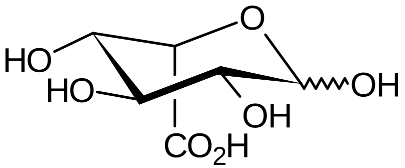
D-xylose
Xyl



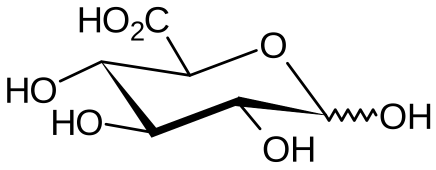
L-rhamnose
Rha



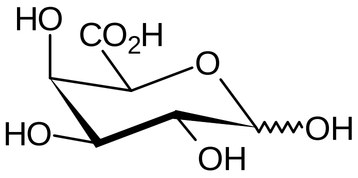
2-amino-2-deoxy-D-glucose
GlcN



L-iduronic acid
IdoA

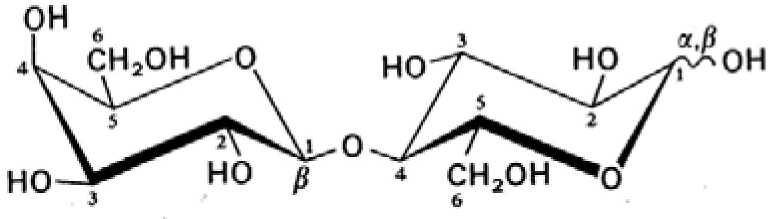


D-glucuronic acid
GlcA

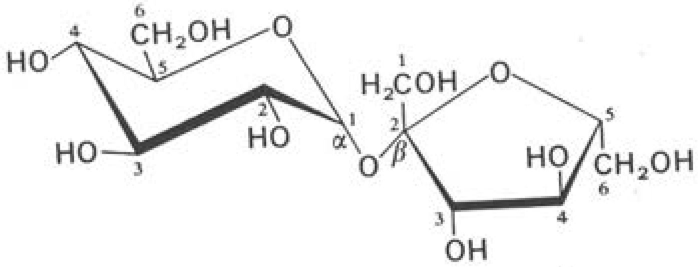


D-galacturonic acid
GalA

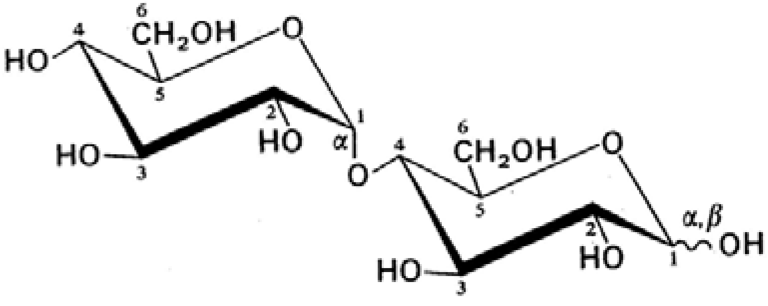
Some Disaccharides



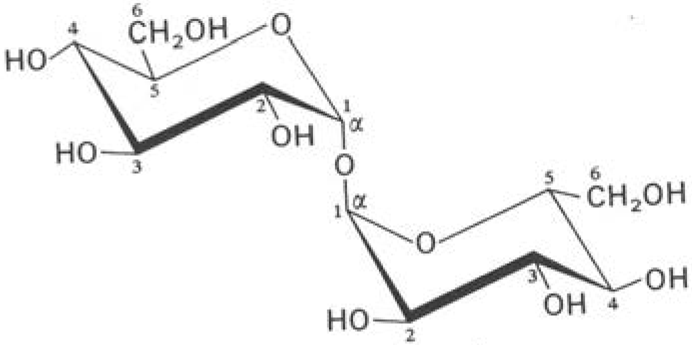
Lactose (Milchzucker) (Gal β(1 → 4) Glc)



Saccharose (Rohrzucker) (Glc α(1 → 2) β Fru)



Maltose (Malzzucker) (Glc α(1 → 4) Glc)



α,α-Trehalose (Glc α(1 → 1) α Glc)

reducing

non-reducing

Structure and function of selected Polysaccharides and Glycoconjugates

Polymer	Typ*	sich wiederholende Einheit	Größe (Zahl der Monosaccharideinheiten	Funktion
Starch Amylose	Homo-	(α1→4)Glc, linear	einige Tausend bis 500 000	Energy storage plants
Amylopectin	Homo-	(α1→4)Glc, mit (α1→6)Glc-Seitenketten alle 24–30 Reste	bis zu 10^6	Energy storage plants
Glycogen	Homo-	(α1→4)Glc, mit (α1→6)Glc-Seitenketten alle 8–12 Reste	Heterogeneous Several millions	Energy storage bacteria, animals
Cellulose	Homo-	(β1→4)Glc	bis zu 15 000	Structure, stability of plant cell walls
Chitin	Homo-	(β1→4)GlcNAc	Very big	Structure, stability of insect exosceleton, spiders crustaceans
Peptidoglycan	Hetero with bound peptides	MurNAc(β1→4)GlcNAc	Very big	Structure, stability of bacterial cell wall
Glycosaminoglycan (Hyaluronat)	Hetero, acidic	GlcUA(β1→3)GlcNAc	Varying ($>10^6$)	Structure, extracellular matrix in skin, connective tissue, viscosity, grease in vertebrate bones
Proteoglycane	Hetero with bound peptides, mainly carbohydrates	Uronsäure (β1→3)-verknüpft mit sulfatiertem Hexosamin	Varying	Structure, elasticity, viscosity, grease in vertebrate bones

* Jedes Polymer wird als Homopolysaccharid (Homo-) oder Heteropolysaccharid (Hetero-) klassifiziert.

Cellulose

Occurrence: Ca. 40% of the carbon in plants (i.e. $10.5 \bullet 10^{10}$ tons) is actually present as cellulose.

The annual regeneration of cellulose by biosynthesis (photosynthesis) is ca. $1.3 \bullet 10^{9}$ tons. One tree generates ca. 14 g of cellulose / day.
Cellulose occurs in the animal kingdom in some tunicates.
In plants, cellulose functions as a fiber component of highly efficient biological compound materials (e.g. wood).

Construction of a typical plant cell wall:
primary cell wall: 8% cellulose, the remaining portion is hemicellulose and pectins
secondary cell wall: 95% cellulose

Annual production of cotton fiber: $20 \bullet 10^{6}$ tons (nearly equals the production of synthetic textile fibers).
Annual production of cellulose for paper and cardboard manufacture: $> 100 \bullet 10^{6}$ tons

Cellulose

In Nature, cellulose almost never occurs pure. Main other components:

- hemicelluloses
- pectins
- lignin

Cotton fiber consists of 94% cellulose. Some bacteria produce highly pure cellulose.

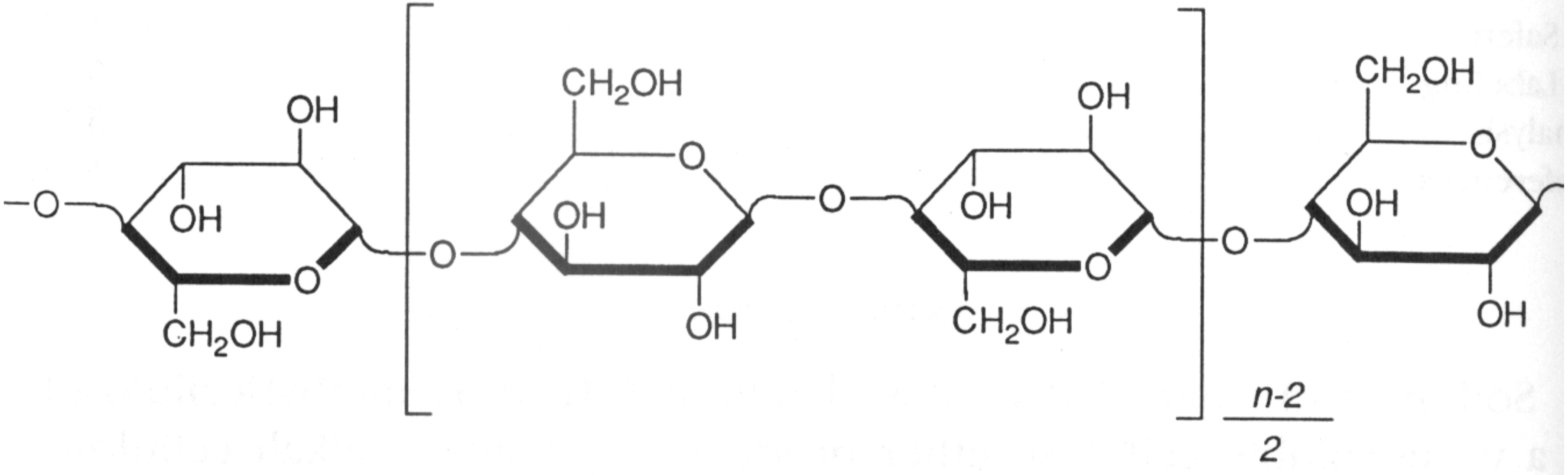


FIG. 1 Cellulose.

Cellulose

Primary structure of cellulose:

hydrolysis with acid → D-glucose

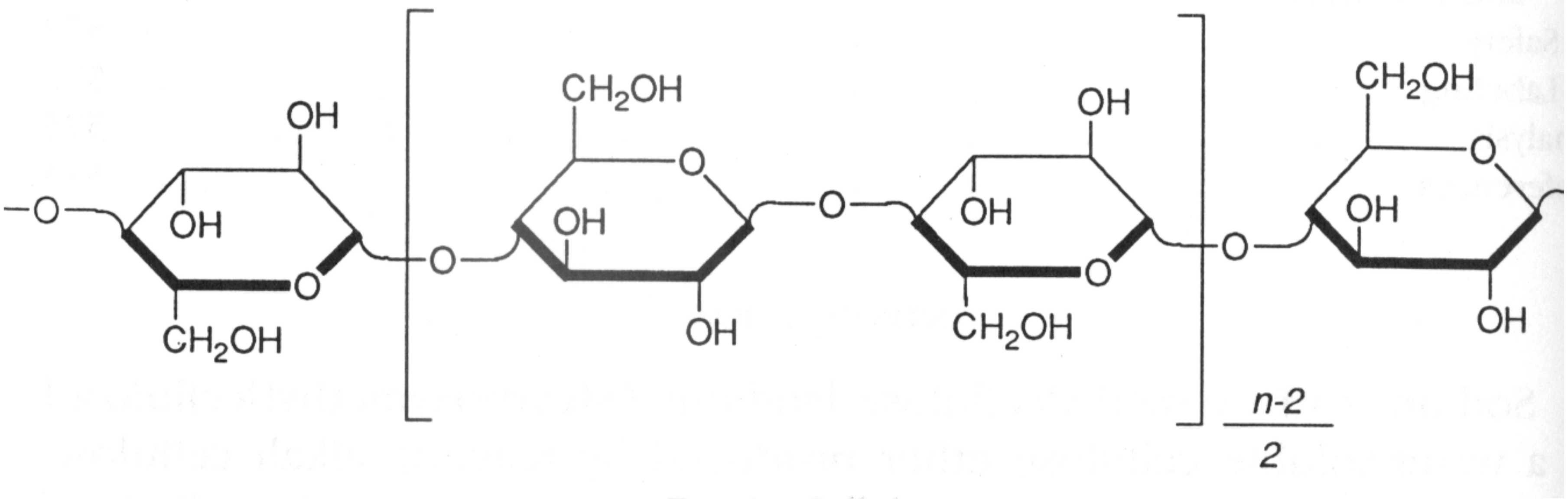
cleavage with β-glucosidase (cellulase) → cellobiose

no cleavage with α-glucosidases

methylation analysis → 2,3,6-tri-*O*-methylglucose + small amounts of 2,3,4,6-tetra-*O*-methylglucose

structure confirmation by X-ray analysis

⇒ cellulose is a syndiotactic polymer of β-D-glucose or an isotactic polymer of cellobiose.



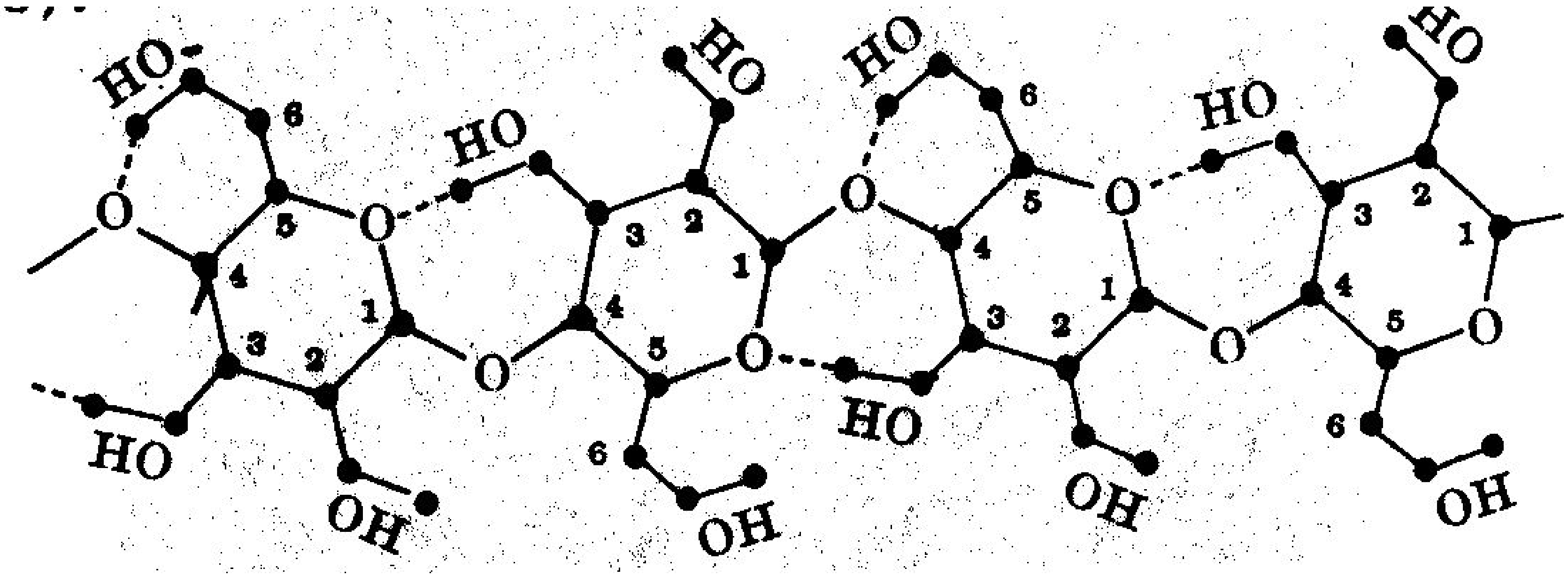
Cellulose

The polysaccharide chain contains one GlcA unit per 500 - 1000 Glc units.

According to P_n (average degree of polymerization; also sometimes abbreviated DP), celluloses are classified as:

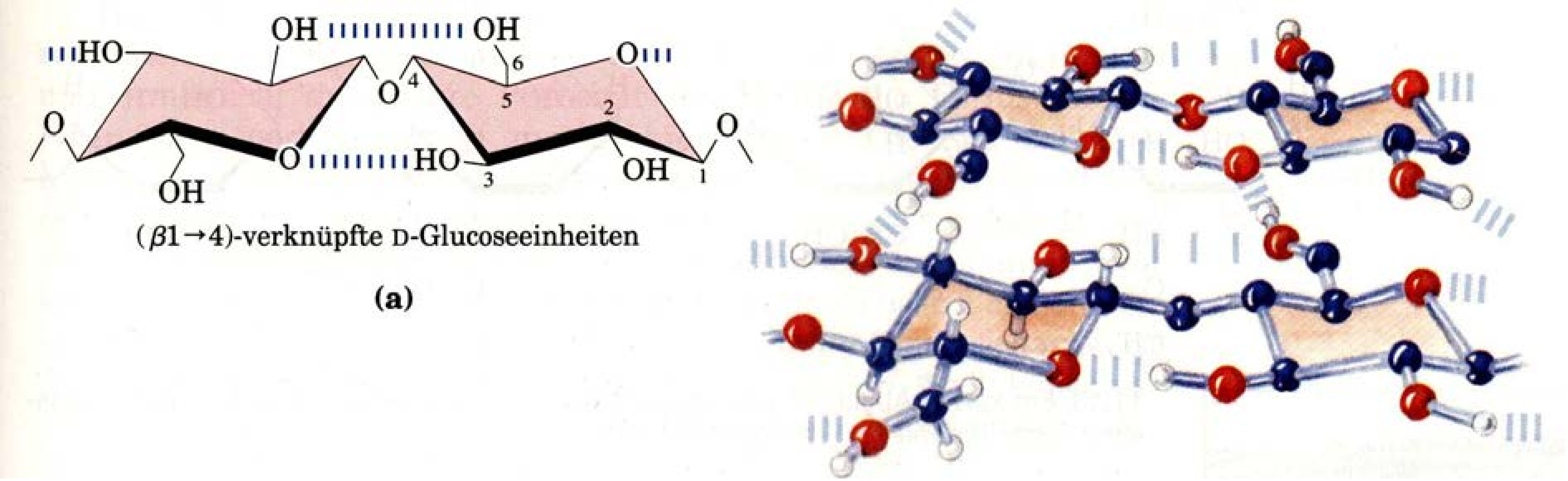
- α-cellulose: $P_n > 150$
- β-cellulose: P_n 10 - 150
- γ-cellulose: $P_n < 10$

P_n varies much (DP 1,000 - 9,000; native cotton fiber cellulose: 10,000 - 14,000) with the source of the cellulose (isolation gives partial degradation, the M_w of native cellulose can only be estimated).



Intrachenar hydrogen bonding in cellulose

Ebert p338



Part of a cellulose chain with hydrogen bonds indicated in blue

- The hydrogen bonds (right drawn to scale) enable a high degree of crosslinking, hydrogen bonds are of superior influence on the molecular structure as in most polysaccharides
- Hydrogen bonding leads to parallel fibers (High stress resistance)
- Compare this structure (β 1-4) to the similar molecule of starch (α 1-4) leading to a hydrogen bond stabilized helix

Secondary structure of cellulose

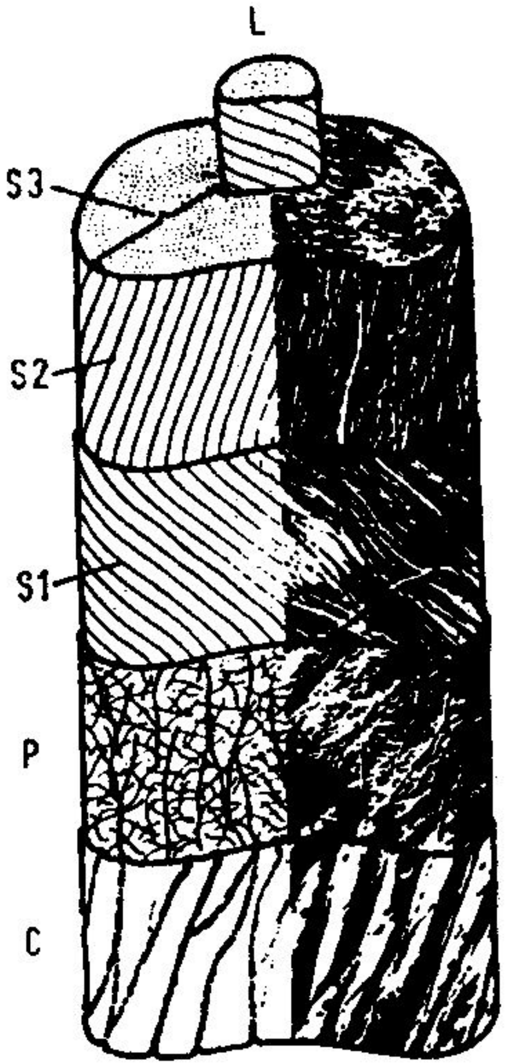
Cotton fiber: polysaccharide chain → elementary fiber → microfiber (Ø 2 - 4 nm) → macrofiber (Ø 300 nm).

In wood, lignin fills the spaces (5 - 10 nm) between the elementary fibers.

Crystalline segments are interrupted by non-crystalline segments: degree of crystallinity in native cellulose: 60% → **cellulose I**

X-ray analysis of cellulose I shows parallel orientation of the polysaccharide chains. Superstructure: helical twist around the b-axis.

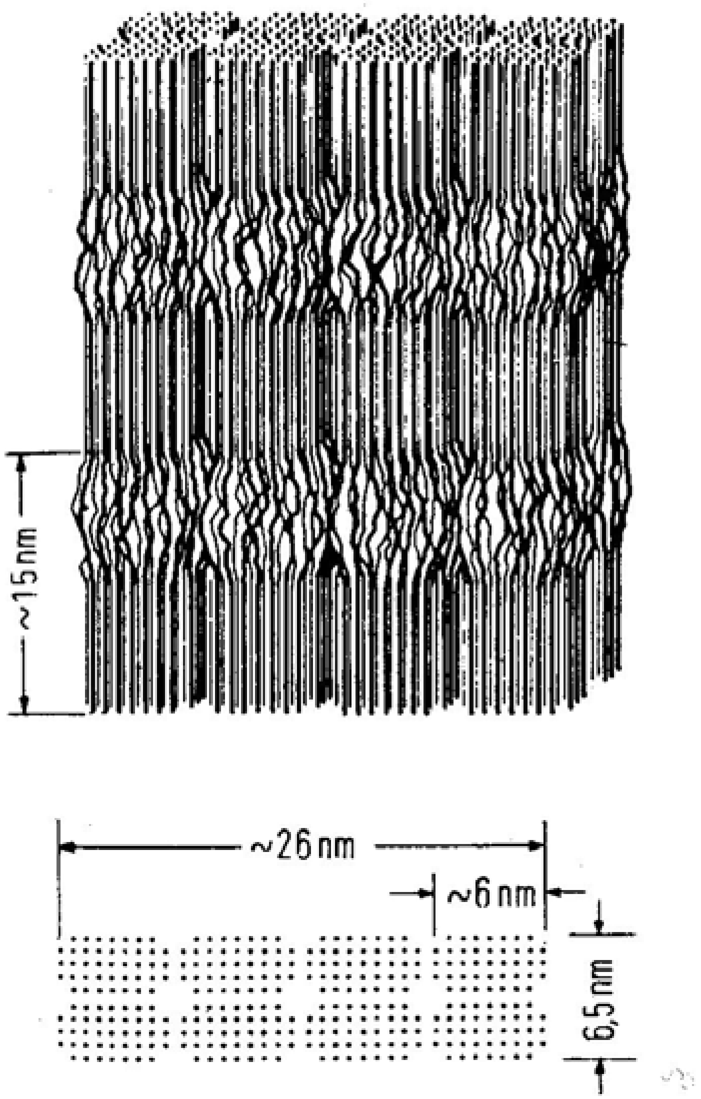
intrachenar H-bonds: -O-4….H….O-6'-, -O-3….H….O-5'- cause insolubility of cellulose in most solvents.



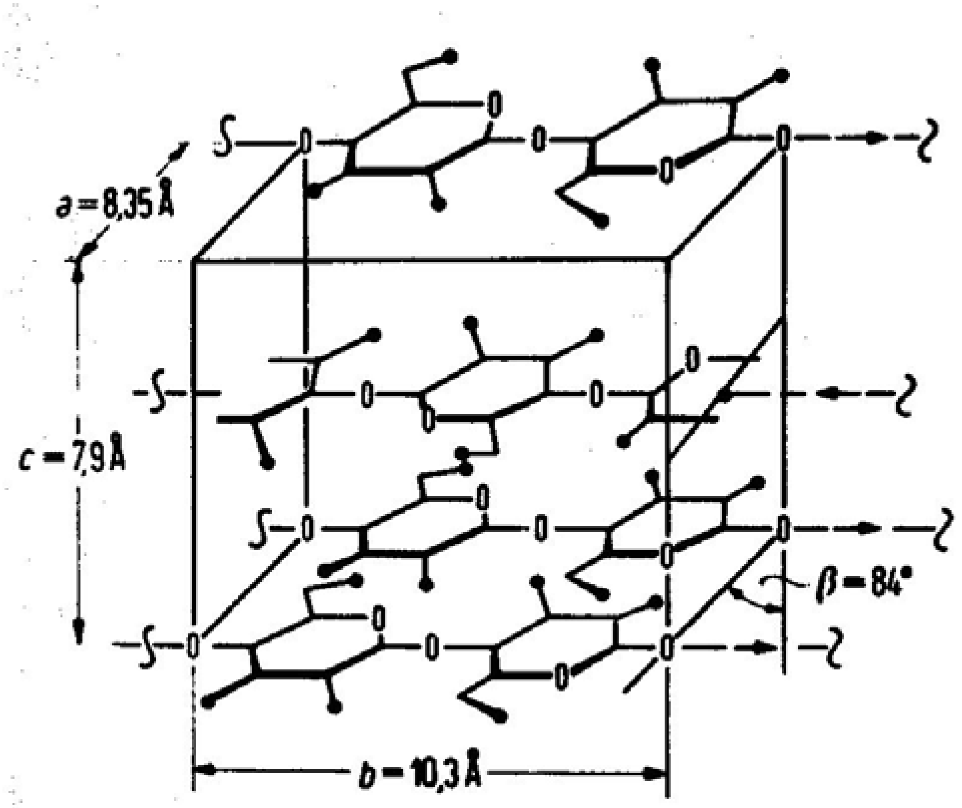
Orientation of cellulose fibers in cotton fiber: C: Cuticle; P: Primary Cell Wall; S1, S2, S3: layers of the secondary cell wall; L: Lumen

SEM of a cell wall from algae: note the parallel orientation of the microfibers

Ebert p335/337



Assembly of a cellulose fiber in algae



X-ray diffraction structure of cellulose I

Ebert p336/337

Cellulose

Solubilization of cellulose, results in partial degradation:

- LiCl / *N*,*N*´-dimethylacetamide
- *N*-methylmorpholine-*N*-oxide / H_2O
- trifluoroacetic acid / halogenalkanes

⇒ lyotropic mesophases

- alkaline Cu(II)tetraminehydroxide (Cuoxam)
- $[Cu(NH_3)_4]^{++}\ SO_4^{--}$
- Cu(II)ethyleneamine hydroxide
- Fe-Na-tartrate

⇒ dissolution by metal complex formation

in addition: **inter**catenar H-bonds

Cellulose

- cellulose I → treatment with conc. NaOH → cellulose II. Cellulose II has antiparallel chain orientation; the transformation is irreversible; cellulose II is thermodynamically more stable than cellulose I.

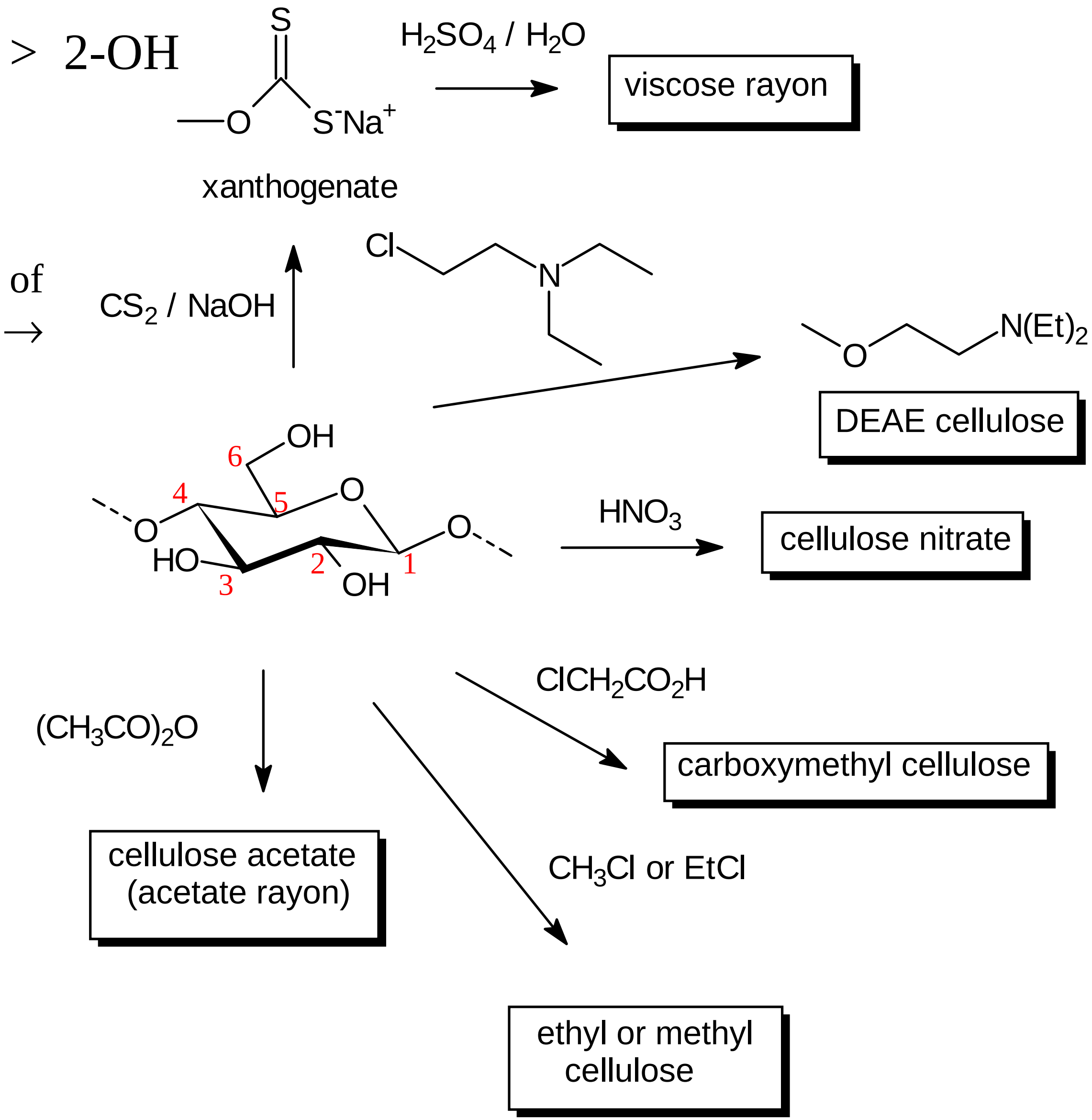
- treatment of cellulose I with 20 - 25% NaOH at 35 – 40 °C under strain is called mercerization → results in increase of stiffness by 30%, glossy appearance, dyeing, wash fastness.

- Other cellulose modifications (IIA, IIB, III, IV) are known, they occur as intermediates in the cellulose I → cellulose II transition; X-ray analysis shows variations in the dimensions of the unit cell.

Cellulose chemistry

Many derivatives of cellulose are known. Most important (see figure):

Reactivity: 6-OH > 3-OH > 2-OH



Some applications:

Xanthogenate

spinning, followed by regeneration of xanthogenate in dilute H_2SO_4 → textiles, cellophane

DEAE cellulose
ion exchanger, chromatography

cellulose nitrate
gun powder, celluloid (mix. with camphor), collophonium

carboxymethyl cellulose
ion exchanger, chromatography, thickener

alkyl cellulose
emulgators, waterproof paper

cellulose acetate
acetate silk, films, plastics

Chemically modified Cellulose

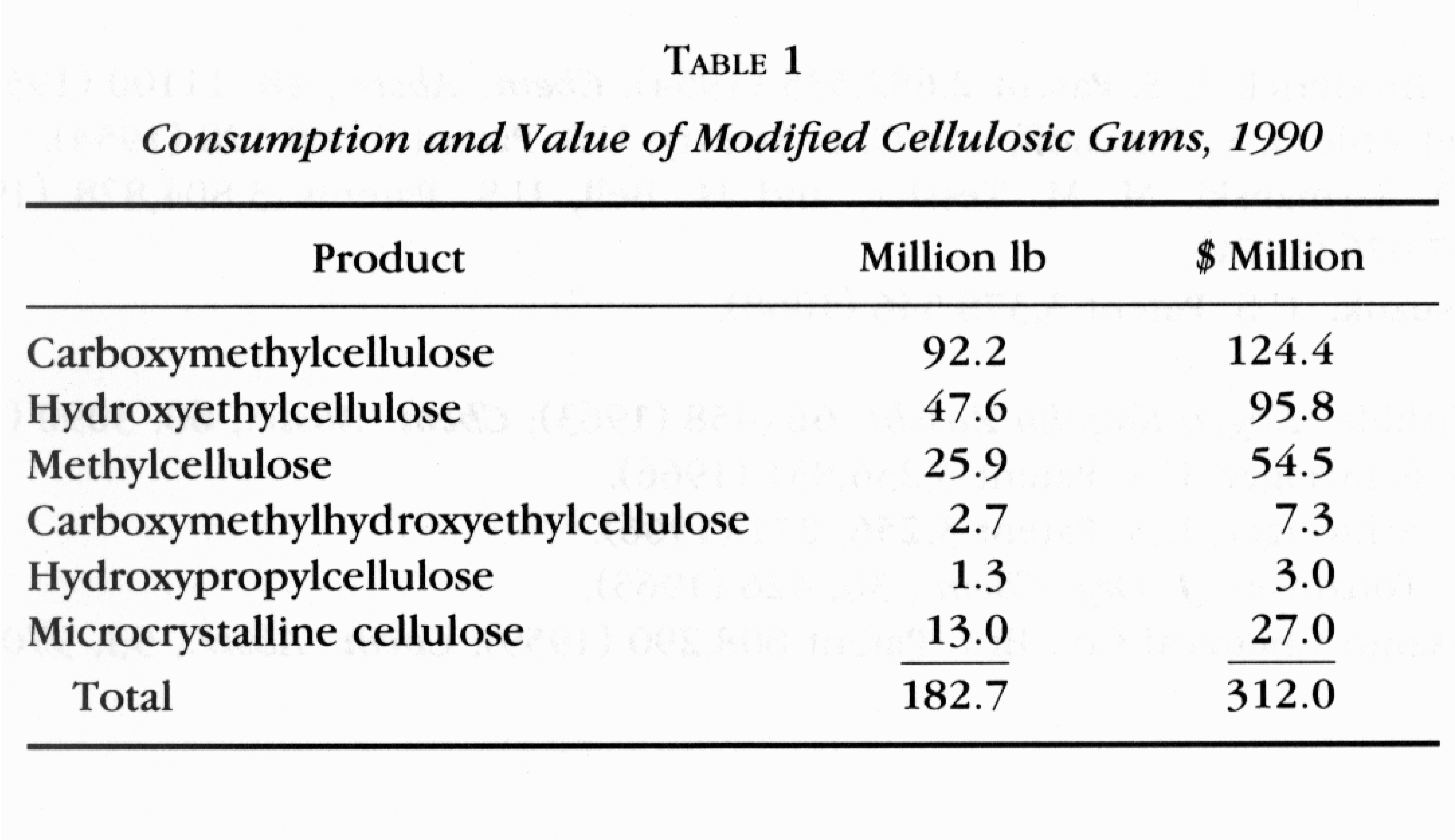


TABLE 1

Consumption and Value of Modified Cellulosic Gums, 1990

Product	Million lb	$ Million
Carboxymethylcellulose	92.2	124.4
Hydroxyethylcellulose	47.6	95.8
Methylcellulose	25.9	54.5
Carboxymethylhydroxyethylcellulose	2.7	7.3
Hydroxypropylcellulose	1.3	3.0
Microcrystalline cellulose	13.0	27.0
Total	182.7	312.0

Source: Industrial Gums Handbook

Carboxymethylcellulose

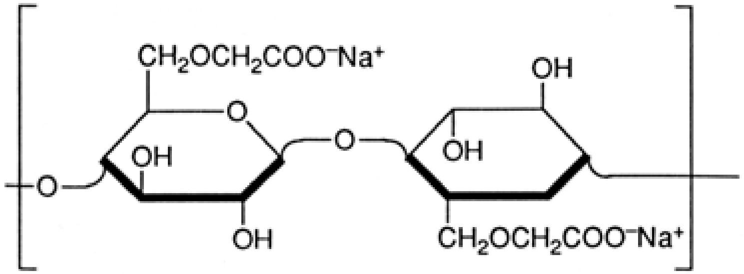


FIG. 2 Idealized structure of sodium carboxymethylcellulose with a DS of 1.0.

Thixotropy

Gel centers tend to produce a three dimensional structure which is broken by shear

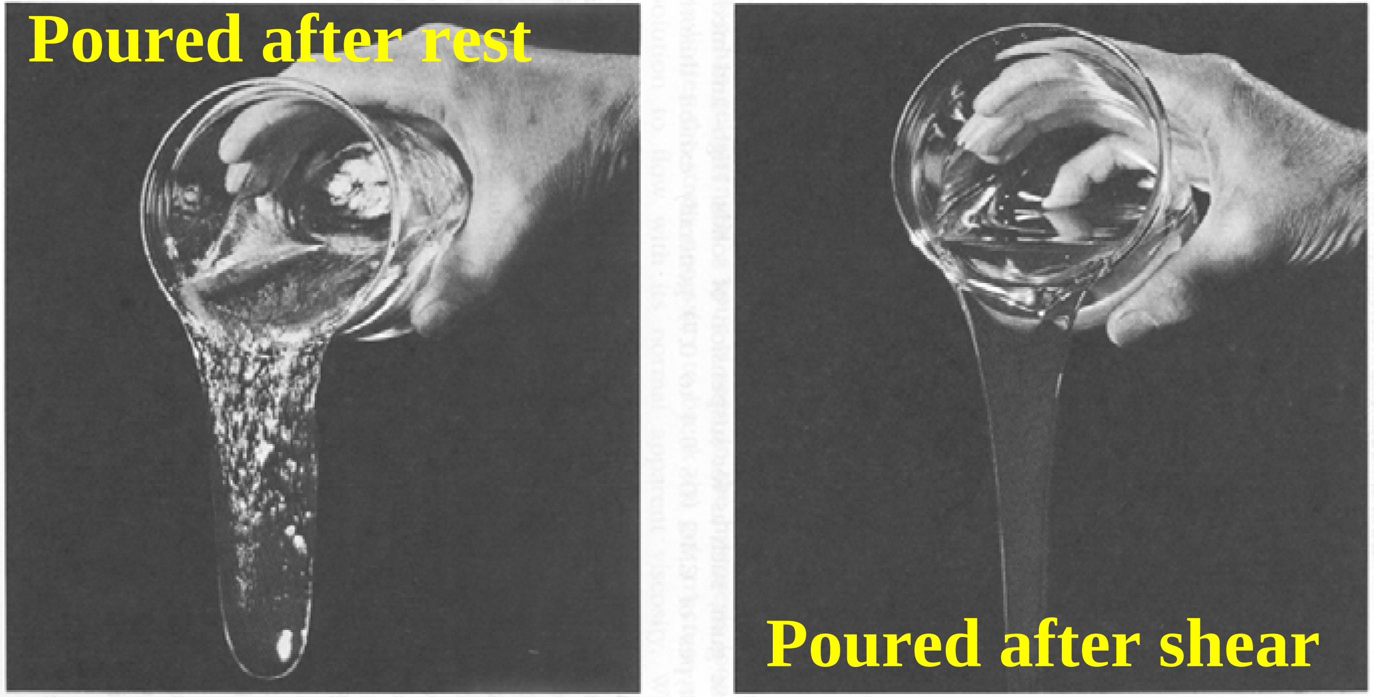


FIG. 10 Left thixotropic and right nonthixotropic solutions of CMC.

Source: Industrial Gums Handbook

Hemicelluloses

These are components of cell walls of plants. Hemicelluloses are soluble in dilute alkali. Annual production $3x10^{10}$ tons (20–30% of cell walls)

Hemicelluloses consist mainly of three polysaccharides:
α- and β-Celluloses are mentioned in Section 2.1.

- **Mannans**

poly(β-1,4-D-mannose). Mannose is a hexose, mannan is therefore a hexosan.

Mw is lower than that of cellulose. Mannans are partially acetylated.

Occurs together with cellulose in plant cell walls.

Occurs in pure form also in some seaweeds which sometimes do not contain cellulose. Mannan is also present in some plant seeds as a storage polysaccharide.

Hemicelluloses

- **Xylans**

poly(β-1,4-D-xylose). Xylose is a pentose, xylan is therefore a pentosan. The polysaccharide is partially acetylated and contains a few branches consisting of L-arabinose and 4-*O*-methylglucuronic acid.

In some algae and seaweeds, the only polysaccharide is poly(β-1,3-xylan)

β-1,4-Xylan is amorphous, β-1,3-xylan is crystalline.

The hemicelluloses of the wood of conifers contain 75% mannan and 25% xylan, those of broad leaf trees contain 25% mannan, 75% xylan.

Chitin and Chitosan

Chitin is poly-(β-1→4-*N*-acetylglucosamine) [poly-(GlcNAc)]. For general references on chitin and chitosan, see some textbooks [10] and conference proceedings [11].

Occurrence: Chitin is a component of the exoskeleton of insects and crustacea as well as in the cell wall of yeasts and fungi where its relative amounts are in the range of 30 to 60%. Actually, there is a constant "rain" of chitin on the ocean floor [12].

Chitin serves as a fibrous element in biological composite materials. Thus, except in some Diatomea, it is always associated with

- proteins which function as the matrix
- polyphenols (in insects [13])
- minerals: predominantly calcium carbonate (calcite) in crustacea.

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[10] R.A.A. Muzzarelli: *Chitin*, Pergamon Press, Oxford, 1977; G.A.F. Roberts, *Chitin Chemistry*, Macmillan, Houndmills, 1992; R.A.A. Muzzarelli, M.G. Peter (eds.), *Chitin Handbook*, Atec, Grottammare, 1997.

[11] R. Muzzarelli, C. Jeuniaux, and G.W. Gooday, Eds., *Chitin in Nature and Technology*, Plenum Press, New York, 1986; G. Skjåk-Bræk, T. Anthonsen, and P. Sandford, Eds., *Chitin and Chitosan*, Elsevier Applied Science, London, 1989; C.J. Brine, P.A. Sandford, and J.P. Zikakis, Eds., *Advances in Chitin and Chitosan*, Elsevier Applied Science, London, 1992; ***Advan. Chitin Sci.,*** Vol. 4, University of Potsdam, 2000.

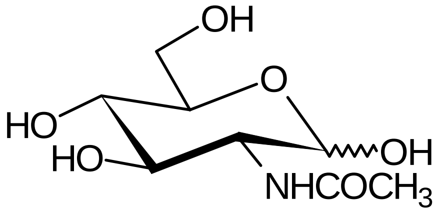
[12] C. Yu, A.M. Lee, B.L. Bassler, and S. Roseman, *J. Biol. Chem.*, **266**, 24260 (1991).

[13] M.G. Peter, *Chem. uns. Zeit*, **27**, 189 (1993).

Chitin and Chitosan

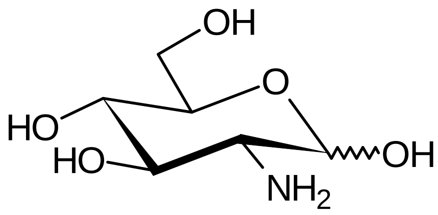
Chitosan is poly-(β-1,4-glucosamine) [poly-(GlcN)]. It occurs naturally in several fungi, esp. *Mucor* species. Chistosan is usually prepared by deacetylation of chitin (see section "Chemistry").

Actually, neiter chitin nor chitosan are pure homopolymers. Chitin nearly always contains some GlcN units and, likewise, chitosan always contains some GlcNAc units. The criteria for distinguishing between chitin and chitosan are the solubilities of the polymers in dilute aqueous acid: chitin is insoluble while chitosan forms viscous solutions. As a rule of thumb, the degree of *N*-acetylation (DA) of chitosan is 40%.



2-acetamido-2-deoxy-D-glucose

GlcNAc

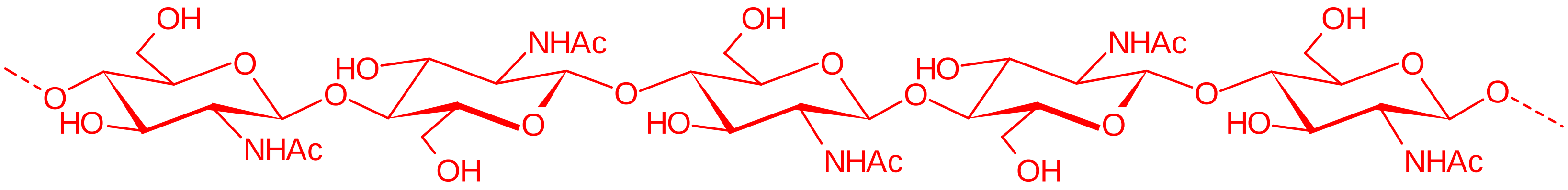


2-amino-2-deoxy-D-glucose

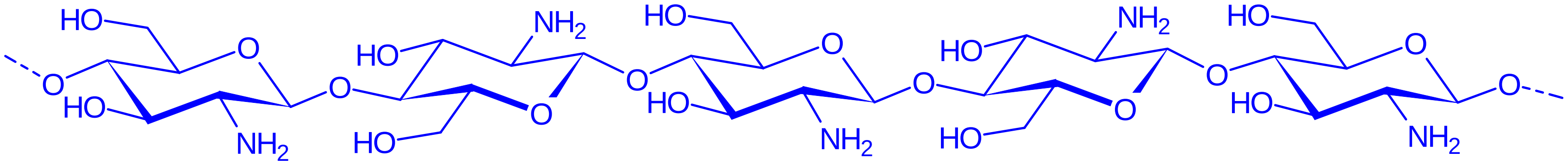
GlcN

Chitin and Chitosan

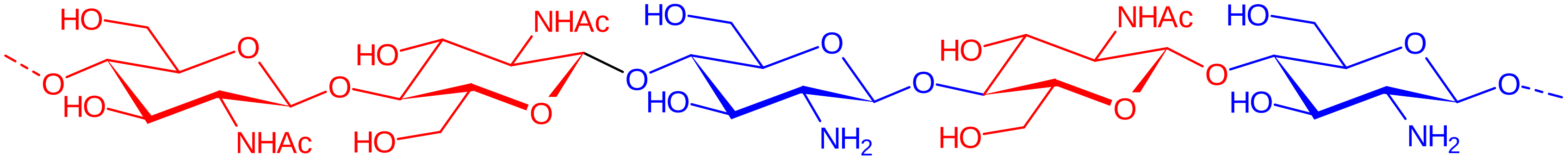
Biotechnological production of chitin is considered, though presently not being economically attractive.



CHITIN



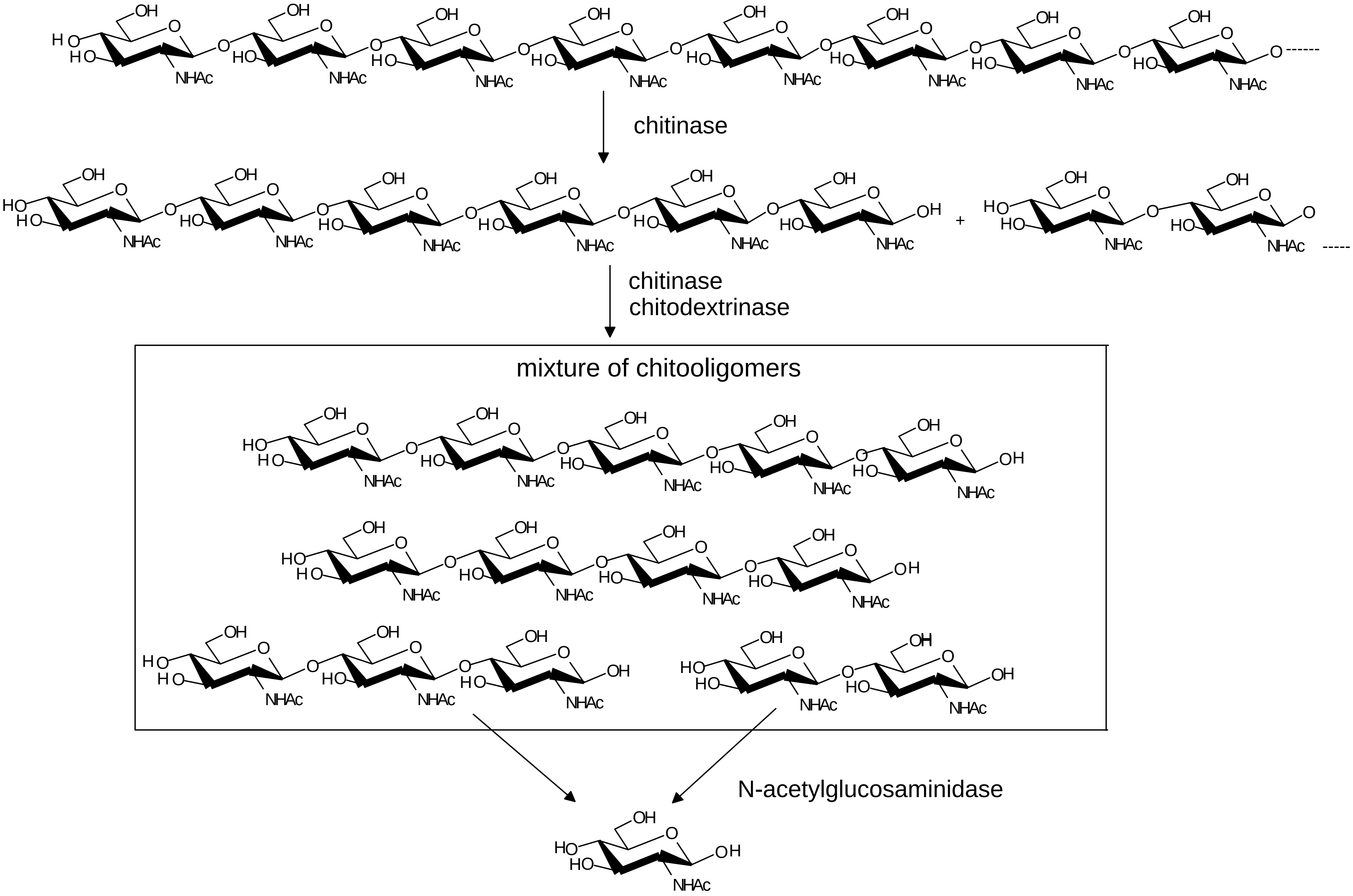
CHITOSAN



CHITIN [F_A 0.60 or CHITOSAN [F_A 0.40

Chitin is soluble in 12 N cold hydrochloric acid or in LiCl/dimethylacetamide (c.f. cellulose), chitosan is soluble in weak acids (acetic acid).

Chitin and Chitosan



chitinase

chitinase
chitodextrinase

mixture of chitooligomers

N-acetylglucosaminidase

Chitin and Chitosan

Primary structure of chitin

The average molecular weight of native chitin as it occurs in the cuticle of insects and crustacea may be estimated from the dimensions of the microfibrils (see below) to be in the order of 1-2 x 10^6 Da.

Hydrolysis of chitin with boiling HCl gives glucosamine and acetic acid.

Secondary structure:

There are close similarities in the structures of chitin and cellulose.

The chitin of insect and crustacean cuticle occurs in the form of microfibrils of typically 10-25 nm in diameter and 2-3 m in length.

Three modifications are known which differ in the orientation of the polysaccharide chains within the microfibrillae, namely α- (antiparallel), β- (parallel), and γ- (two parallel, one antiparallel) chitin. The most abundant form is α-chitin.

Chitin and Chitosan

X-ray analysis of chitin and chitin-protein complexes show that the microfiber is packed into a matrix of helically arranged proteins [22]. The association is stabilized by hydrogen bonding but also by salt formation between protonated free amino groups of the polysaccharide and carboxylate groups of the polypeptide.

Mechanical properties: The Young's modulus of elasticity of chitin fibrils of locust tendon (E = 70 - 90 GPa) is comparable with that of gold.

In biological materials (e.g. different types of insect or spider cuticles, or tendons of arthropods), chitin fibers show varying orientations:

- parallell: stiff materials such as locust tendon
- parallel layers in block-like arrangements or helicoidal orentation: elastic materials; plywood effect

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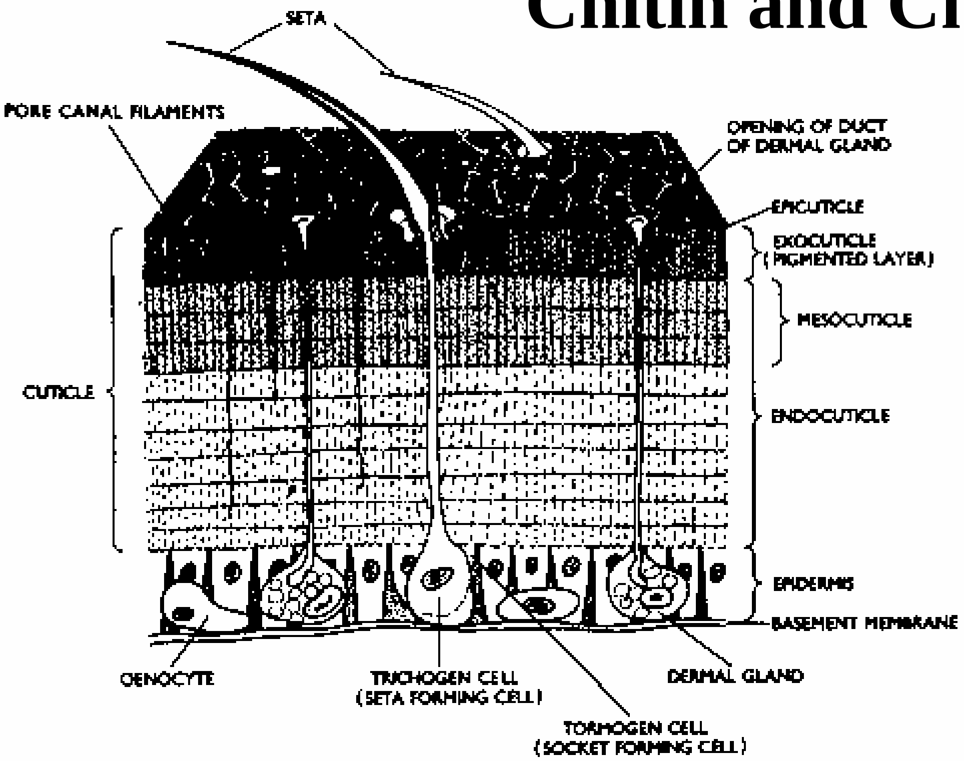
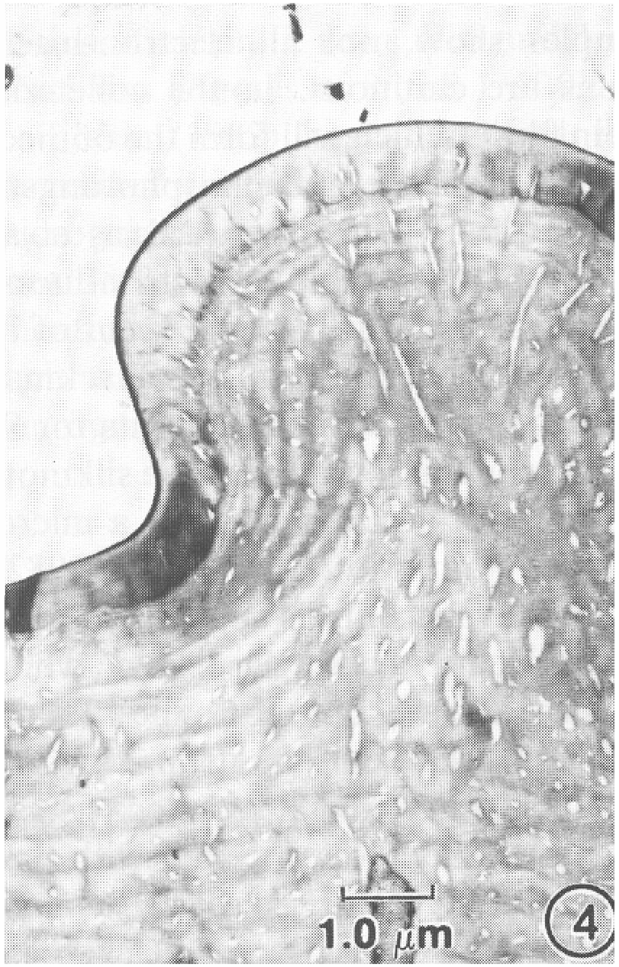
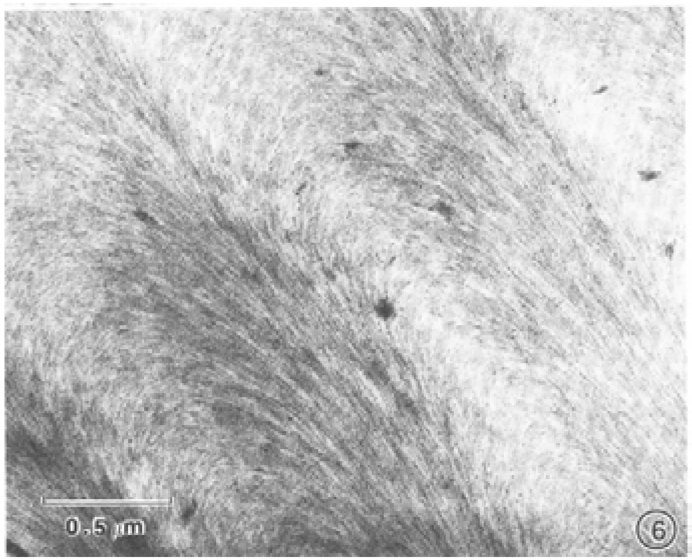
[22] J. Blackwell, M.A. Weigh, The structure of chitin-protein complexes, in: J.P. Zikakis (ed.), Chitin, Chitosan, and Related Enzymes, Academic Press, New York, 1984, pp. 257-272.

Chitin and Chitosan

Insect Cuticle



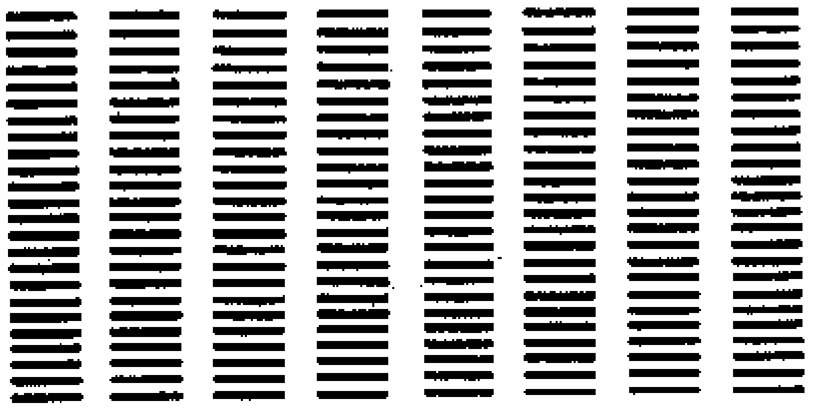




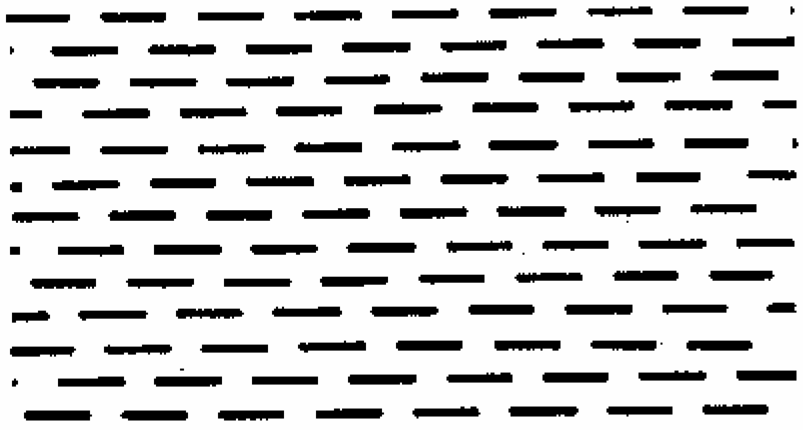
Hackman, R. H. (1987) Chitin and the fine structure of insect cuticles, in: *Chitin and Benzoylphenyl Ureas* (Wright, J. E., Retnakaran, A., Eds.), pp. 1-32. Dordrecht: W. Junk.

Chitin and Chitosan

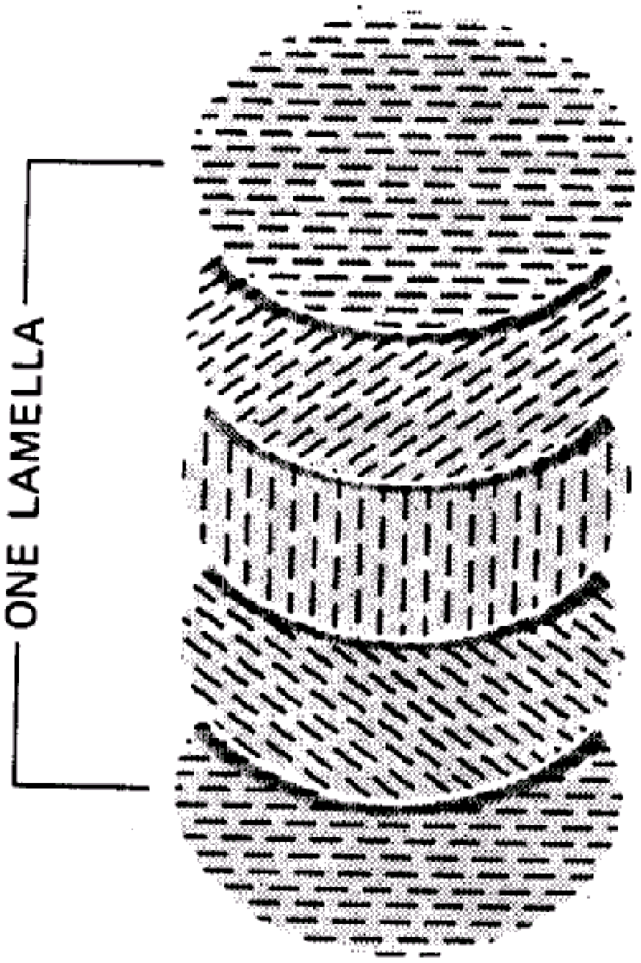
Liquid Crystal Properties of Insect Cuticle Chitin



smectic



nematic



cholesteric

Hackman, R. H. (1987) Chitin and the fine structure of insect cuticles, in: *Chitin and Benzoylphenyl Ureas* (Wright, J. E., Retnakaran, A., Eds.), pp. 1-32. Dordrecht: W. Junk.

Chitin and Chitosan

Liquid crystals and cuticle *as a* ***liquid crystal analogue***

Liquid crystals are highly organized geometric systems (Gray 1962). They are ordered liquids, being neither crystalline solids nor amorphous liquids. They consist of elongated molecules and, in the absence of bulk flow, show birefringence. Liquid crystals may exist in one of three basic states or mesomorphic phases, viz. **smectic, nematic or cholesteric**. Changes in temperature or in concentration may bring about a change in the phase adopted.

In the smectic phase the molecules are arranged in parallel layers, the heads and tails of all molecules being alligned, i.e. there is order in the direction of the molecular axes and in the position of the molecules. In the nematic phase the aligned molecules are arranged unidirectionally but there is no regular arrangement of the ends of the molecules. This represents a lower degree of order than that in the smectic phase. In the cholesteric phase the molecules are arranged in layers and within each layer there is a parallel alignment of molecules. Successive layers are displaced so that the molecular axes trace out a helix.

Chitin and Chitosan

Production of chitin: The most important sources of chitin are the large amounts of waste crab and krill shells from the fishing industry,

Crude chitin:

- crab shells are decalcified at ambient temperature by means of dilute aqueous hydrochloric acid followed by extensive washing with water
- deproteination is achieved with dilute sodium hydroxide.
- Pigments, such as carotenoids, may be extracted with appropriate organic solvents.
- Highly pure chitin can be obtained by adding an ice-cold solution of chitin in 12 N hydrochloric acid slowly to a vigorously stirred large volume of water. This procedure may be repeated several times.
- Calcium carbonate (as a major component of crab shells, is converted to calcium oxide and sodium carbonate.

Chitin and Chitosan

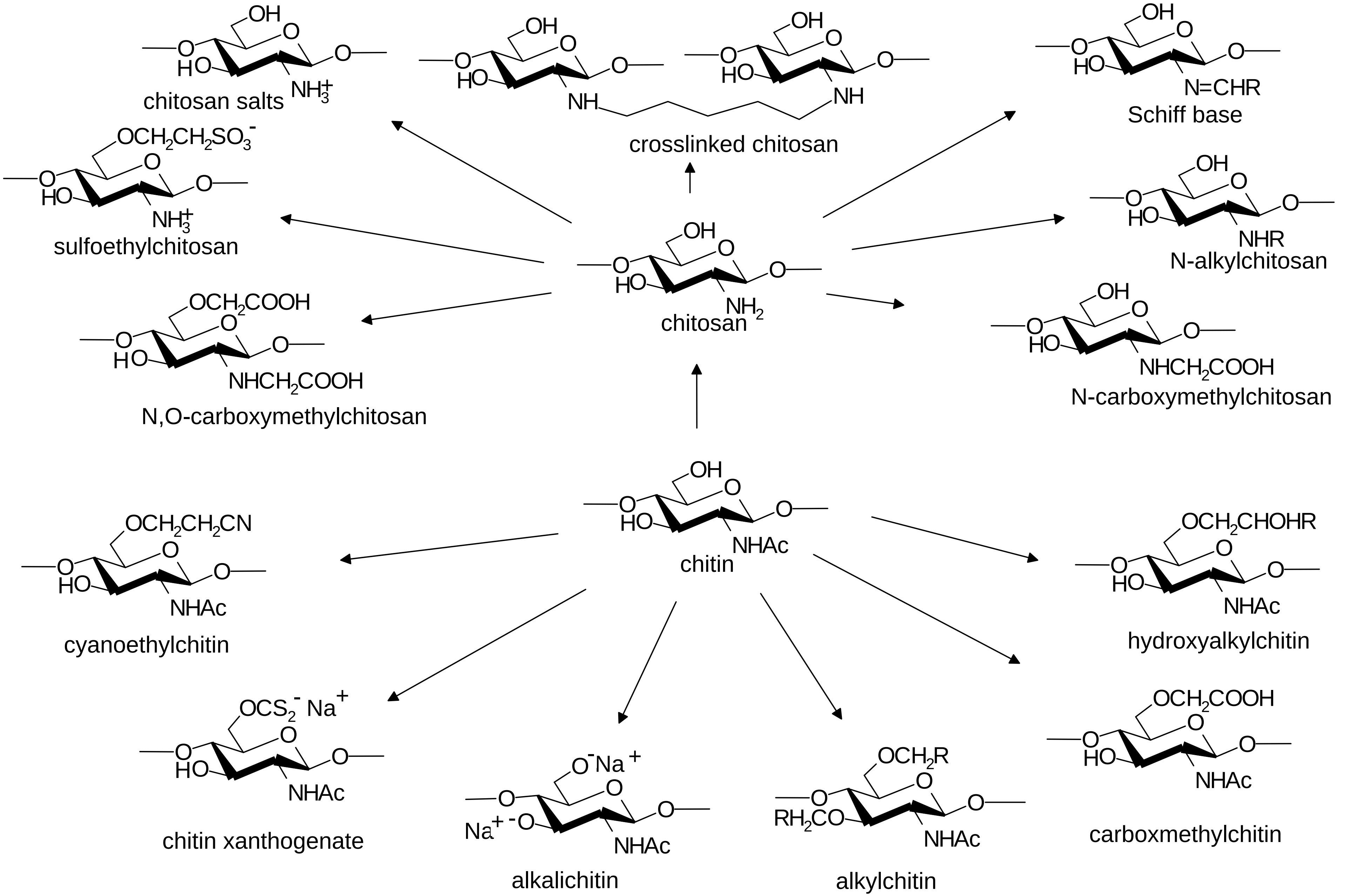
Technical production of chitosan

Step	Reagent	Temperature	Time
Deproteinization	0.5 - 15 % NaOH	25 - 100 °C	0.5 - 72 h
Demineralization	2.5 - 8 % HCl	15 - 30 °C	0.5 - 48 h
Decolouration	various org. solvents; NaOCl, H_2O_2	20 - 30 °C	washing - 60 min
Deacetylation	39 - 60 % NaOH	60 - 150 °C	0.5 - 144 h

The acetyl groups of chitosan may be recovered as sodium acetate.

Commercial preparations of chitosan possess Mw values between 10^4 and 10^5 Da, though higher moleculer weight materials are available, too.

Derivatives of Chitin and Chitosan



chitosan salts

crosslinked chitosan

Schiff base

sulfoethylchitosan

N-alkylchitosan

chitosan

N,O-carboxymethylchitosan

N-carboxymethylchitosan

chitin

cyanoethylchitin

hydroxyalkylchitin

chitin xanthogenate

alkalichitin

alkylchitin

carboxmethylchitin

Summary of chitosan applications

Application	**Properties of Chitosan utilized**
Technical	
Water engineering: Adsorption of metal ions and dyes, flocculation of proteins	Polycation; metal ion complexation; biodegradability
Textiles, fibers, nonwoven fabrics, leather	Polycation; film formation, antibacterial properties
Paper coating	Complex formation with polyanions and polysaccharides
Biotechnology: Enzyme immobilization, plant culture medium supplement, cell encapsulation, protein purification	Chemical functionality

Chitosan applications

Application	**Properties of Chitosan utilized**
Medicine and health care	
Lowering of serum lipids	Polycation; lipid complexation
Bone regeneration; treatment of rheumatoid diseases	Osteoconductivity, GAG synthesis regulation
Vascular medicine and surgery, wound care, artificial skin, hemostasis	Tissue adhesion; haemostatic antibacterial; biological activity on cells
Pharmaceutical	
Sustained release formulations, transmucosal drug delivery; drug targeting	Polyelectrolyte, mucoadhesive

Chitosan applications

Application	**Properties of Chitosan utilized**
Cosmetics	
Skin moisturing ingredient; hair shampoos, hair styling, dentrifices	Gel and film formation; antibacterial
Agriculture	
Plant growth regulators; elicitors of plant defense, seed conservation, soil fertilizer, anti-fungals and anti-nematodals	Plant growth regulator; regulation of resistance proteins; stimulation of chitinase producing soil bacteria

Further polysaccharides from plants and Microorganisms

Thickeners and stabilizers (1988, USA): 280.000 tons, 80 million $

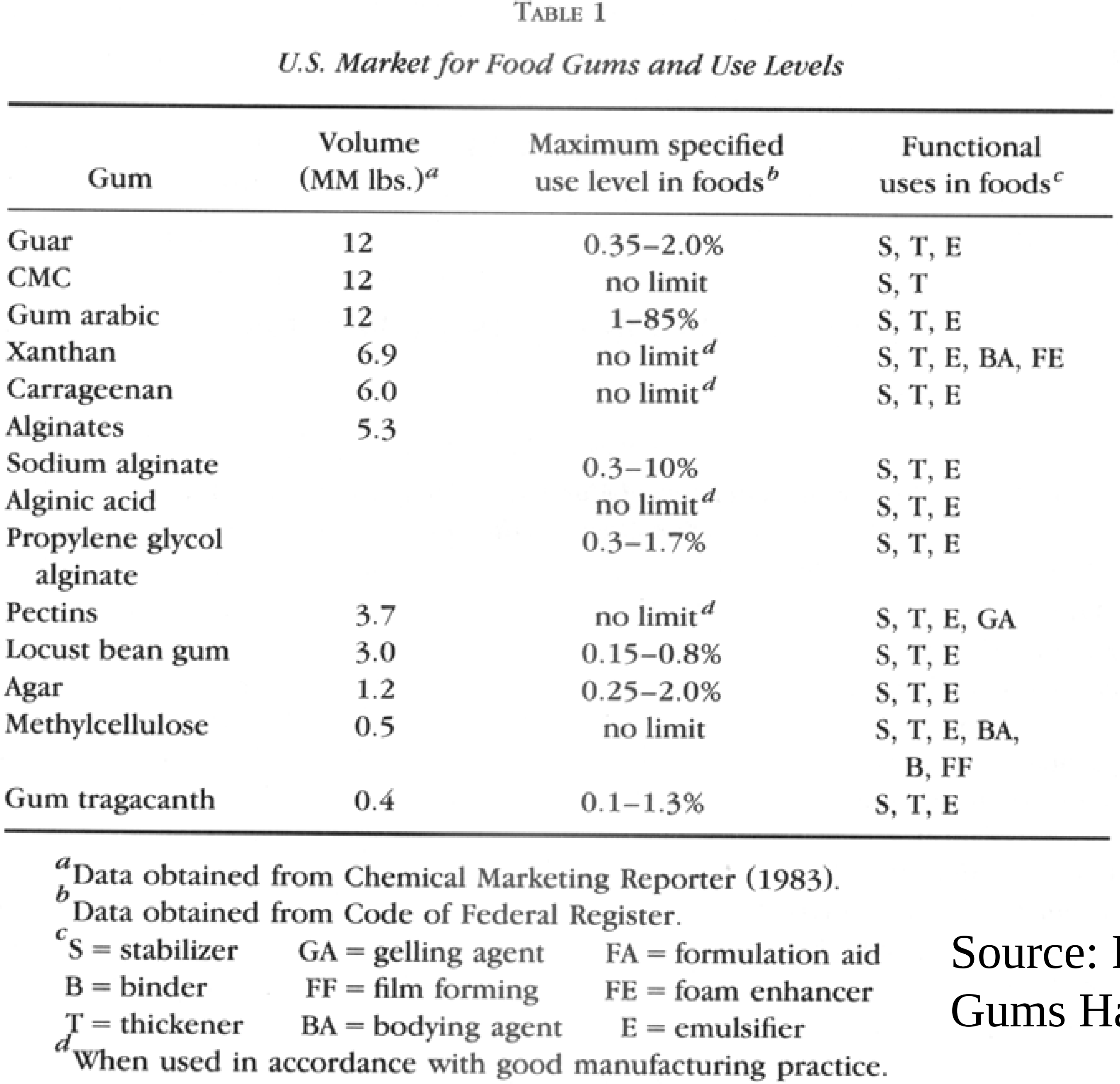


TABLE 1

U.S. Market for Food Gums and Use Levels

Gum	Volume (MM lbs.)[a]	Maximum specified use level in foods[b]	Functional uses in foods[c]
Guar	12	0.35–2.0%	S, T, E
CMC	12	no limit	S, T
Gum arabic	12	1–85%	S, T, E
Xanthan	6.9	no limit[d]	S, T, E, BA, FE
Carrageenan	6.0	no limit[d]	S, T, E
Alginates	5.3		
Sodium alginate		0.3–10%	S, T, E
Alginic acid		no limit[d]	S, T, E
Propylene glycol alginate		0.3–1.7%	S, T, E
Pectins	3.7	no limit[d]	S, T, E, GA
Locust bean gum	3.0	0.15–0.8%	S, T, E
Agar	1.2	0.25–2.0%	S, T, E
Methylcellulose	0.5	no limit	S, T, E, BA, B, FF
Gum tragacanth	0.4	0.1–1.3%	S, T, E

[a] Data obtained from Chemical Marketing Reporter (1983).

[b] Data obtained from Code of Federal Register.

[c] S = stabilizer GA = gelling agent FA = formulation aid
B = binder FF = film forming FE = foam enhancer
T = thickener BA = bodying agent E = emulsifier

[d] When used in accordance with good manufacturing practice.

Source: Industrial Gums Handbook

Further polysaccharides from plants and Microorganisms

- **Pectins**

poly(α-1,4-D-galacturonic acid) where 20-75% of the carboxy groups are methyl esters; (therefore, pectins are copolymers of GalA and GalAMe). DP: 160 - 2800.

Pure pectins occurs in citrus fruits. Other pectins contain branched arabinans and linear galactans. Pectins of sugar beets are partially acetylated.

Pectins are widely distributed in plants in the intercellular space. Rich source of pectins: citrus fruits (up to 30%) and sugar beets (25%) of dry matter.

Pectins

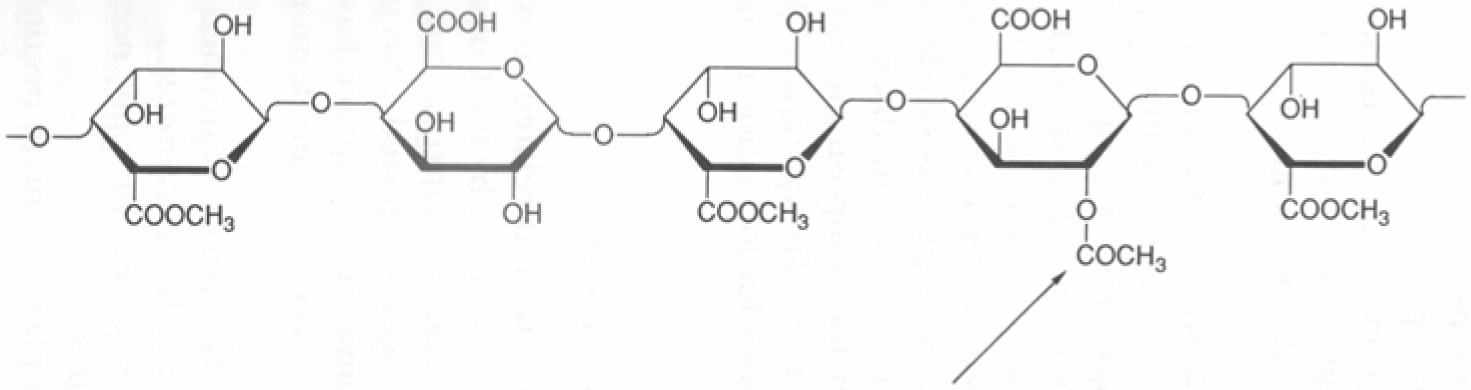


FIG. 1 Section of a pectin molecule with methyl esterified and nonesterified carboxyl groups. The *O*-2 acetyl group at the arrow is rare or absent in commercial pectins.

Pectins are anionic ion exchangers.

In solution: stretched conformation causes highly viscous solutions.

Most pectins form gels → application in food technology: e.g. fruit jelly. Gelation is facilitated by calcium ions (at least 14 GalA units are necessary), lowering pH in highly esterified pectins, addition of saccharose.

Pectin gelation

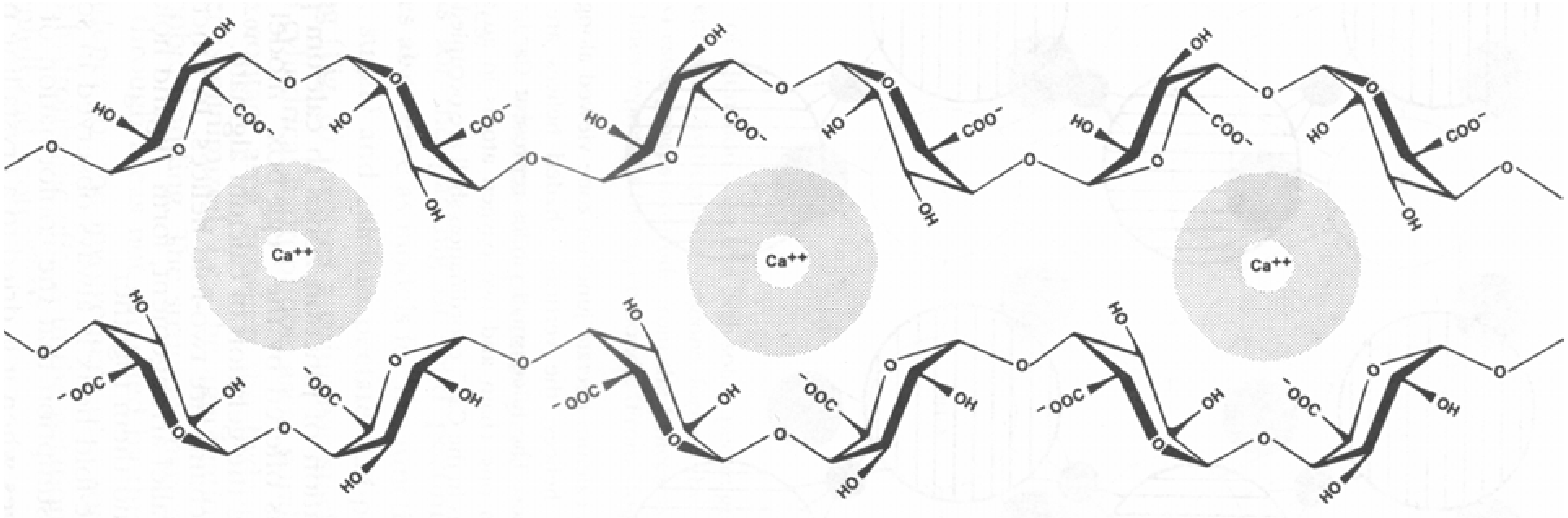


FIG. 10 "Egg box" model of a junction zone in a calcium pectate gel. (From Højgaard Christensen.[x101])

Pectin applications

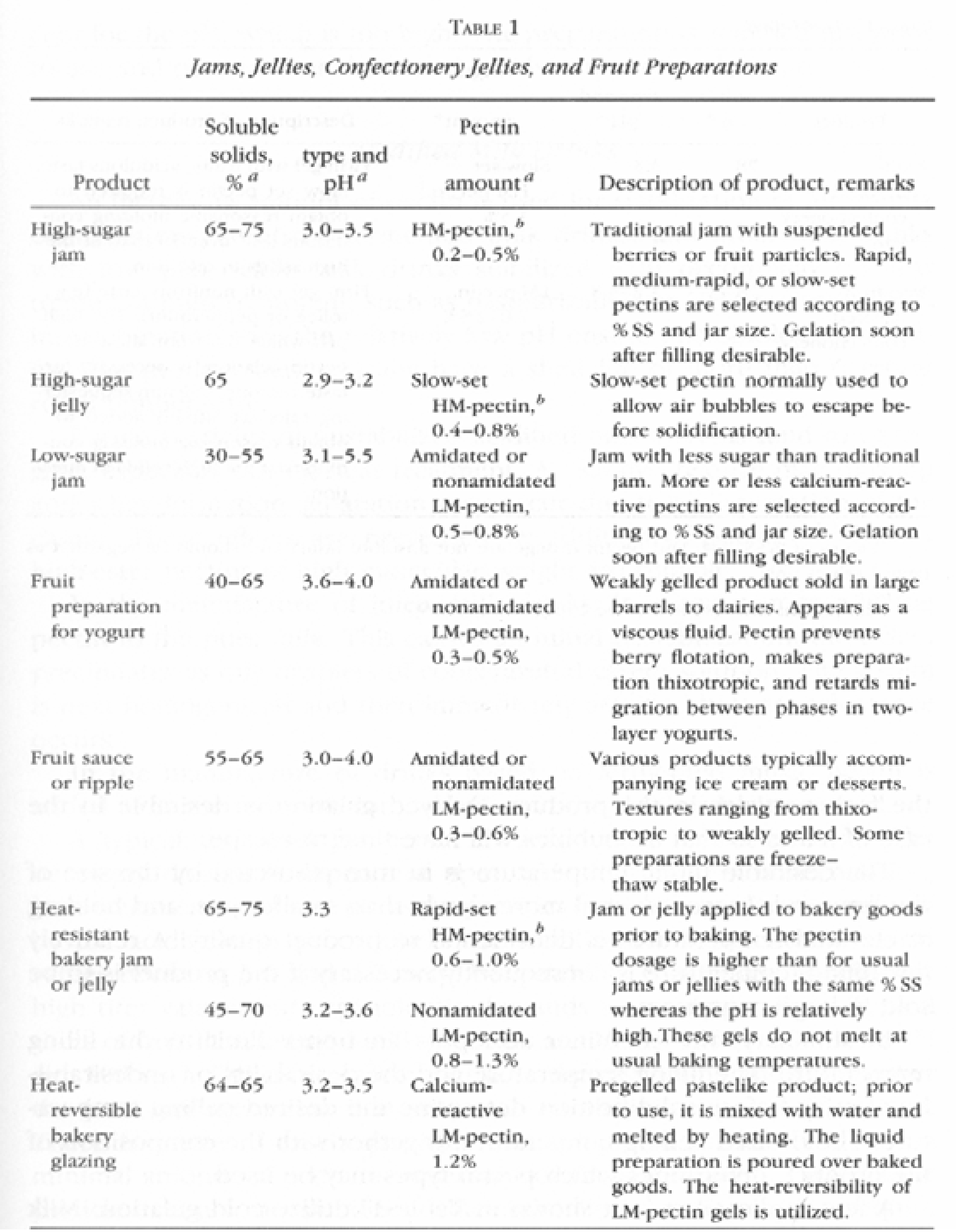


TABLE 1

Jams, Jellies, Confectionery Jellies, and Fruit Preparations

Product	Soluble solids, %[a]	type and pH[a]	Pectin amount[a]	Description of product, remarks
High-sugar jam	65–75	3.0–3.5	HM-pectin,[b] 0.2–0.5%	Traditional jam with suspended berries or fruit particles. Rapid, medium-rapid, or slow-set pectins are selected according to % SS and jar size. Gelation soon after filling desirable.
High-sugar jelly	65	2.9–3.2	Slow-set HM-pectin,[b] 0.4–0.8%	Slow-set pectin normally used to allow air bubbles to escape before solidification.
Low-sugar jam	30–55	3.1–5.5	Amidated or nonamidated LM-pectin, 0.5–0.8%	Jam with less sugar than traditional jam. More or less calcium-reactive pectins are selected according to % SS and jar size. Gelation soon after filling desirable.
Fruit preparation for yogurt	40–65	3.6–4.0	Amidated or nonamidated LM-pectin, 0.3–0.5%	Weakly gelled product sold in large barrels to dairies. Appears as a viscous fluid. Pectin prevents berry flotation, makes preparation thixotropic, and retards migration between phases in two-layer yogurts.
Fruit sauce or ripple	55–65	3.0–4.0	Amidated or nonamidated LM-pectin, 0.3–0.6%	Various products typically accompanying ice cream or desserts. Textures ranging from thixotropic to weakly gelled. Some preparations are freeze–thaw stable.
Heat-resistant bakery jam or jelly	65–75	3.3	Rapid-set HM-pectin,[b] 0.6–1.0%	Jam or jelly applied to bakery goods prior to baking. The pectin dosage is higher than for usual jams or jellies with the same % SS whereas the pH is relatively high.These gels do not melt at usual baking temperatures.
	45–70	3.2–3.6	Nonamidated LM-pectin, 0.8–1.3%	
Heat-reversible bakery glazing	64–65	3.2–3.5	Calcium-reactive LM-pectin, 1.2%	Pregelled pastelike product; prior to use, it is mixed with water and melted by heating. The liquid preparation is poured over baked goods. The heat-reversibility of LM-pectin gels is utilized.

Further polysaccharides from plants and Microorganisms

- **Alginic acids / Alginate**

β-1,4 linked copolymers of D-mannuronic acid and L-guluronic acid. Many different types exist: blockpolymers $(ManA)_n$-$(GulA)_m$; copolymers $(ManA\text{-}GulA)_n$

Occur in algae (Phaeophyceae, brown algae) and seaweeds.
Bind 200 - 300 fold weight of water → gel formation

Insoluble in cold water, Na- and Mg-salts are soluble in water, precipitation

Many applications in the food industry: thickeners in fruit jellies, marmelades, ice cream, etc.

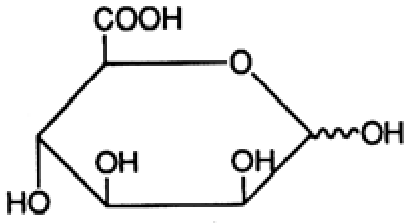
Esters: sugar-O-COR: alginylesters; sugar-CO-OR: alginates. Propylene glycol esters of alginic acids are used as foam stabilizers.

Alginate molecular structure

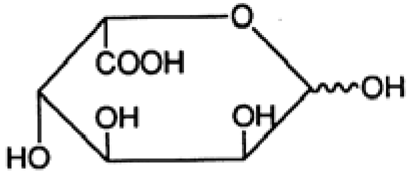
Alginate is composed of two building blocks of monomeric units, namely β-D-mannuronopyranosyl and α-L-guluronopyranosyl units

Ratio of D-mannuronic acid and L-guluronic acid and their sequence determines the alginate properties

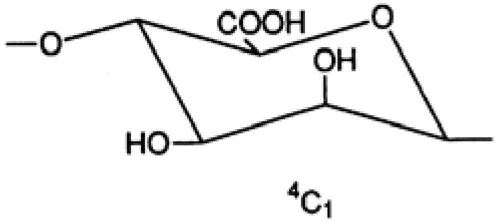
Monomers occur in blocked sequences (M & G blocks)



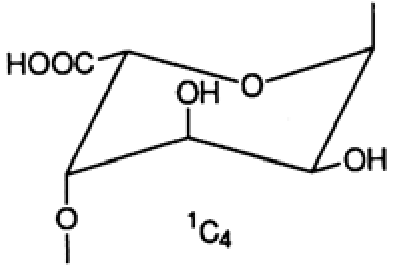
D-Mannuronic acid



L-Guluronic acid



1,4-Linked
β-D-Mannopyranosyluronic acid unit



1,4-Linked
α-L-Gulopyranosyluronic acid unit

Alginate molecular structure

ALGIN 111

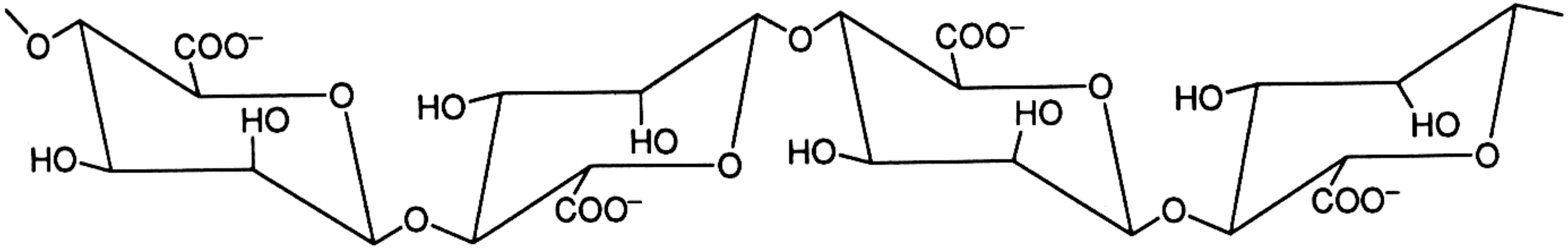


FIG. 4 Block of β-(1 → 4)-linked D-mannuronic acid units.

Alginates form gels with divalent and polyvalent ions (exception magnesium)

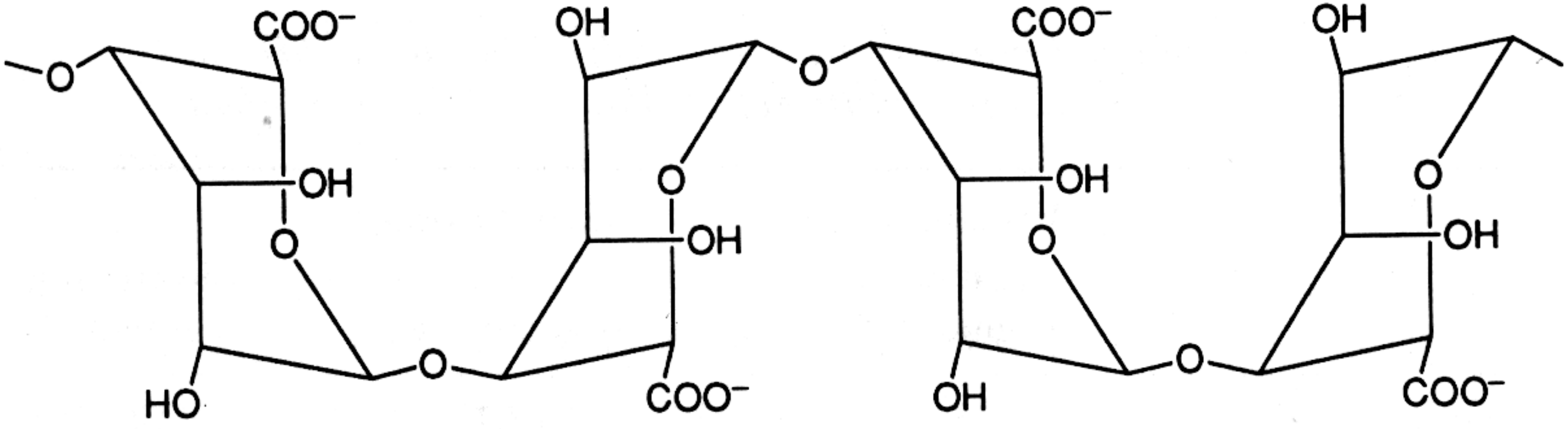


FIG. 5 Block of α-(1 → 4)-linked L-guluronic acid units.

Alginate manufacturing process

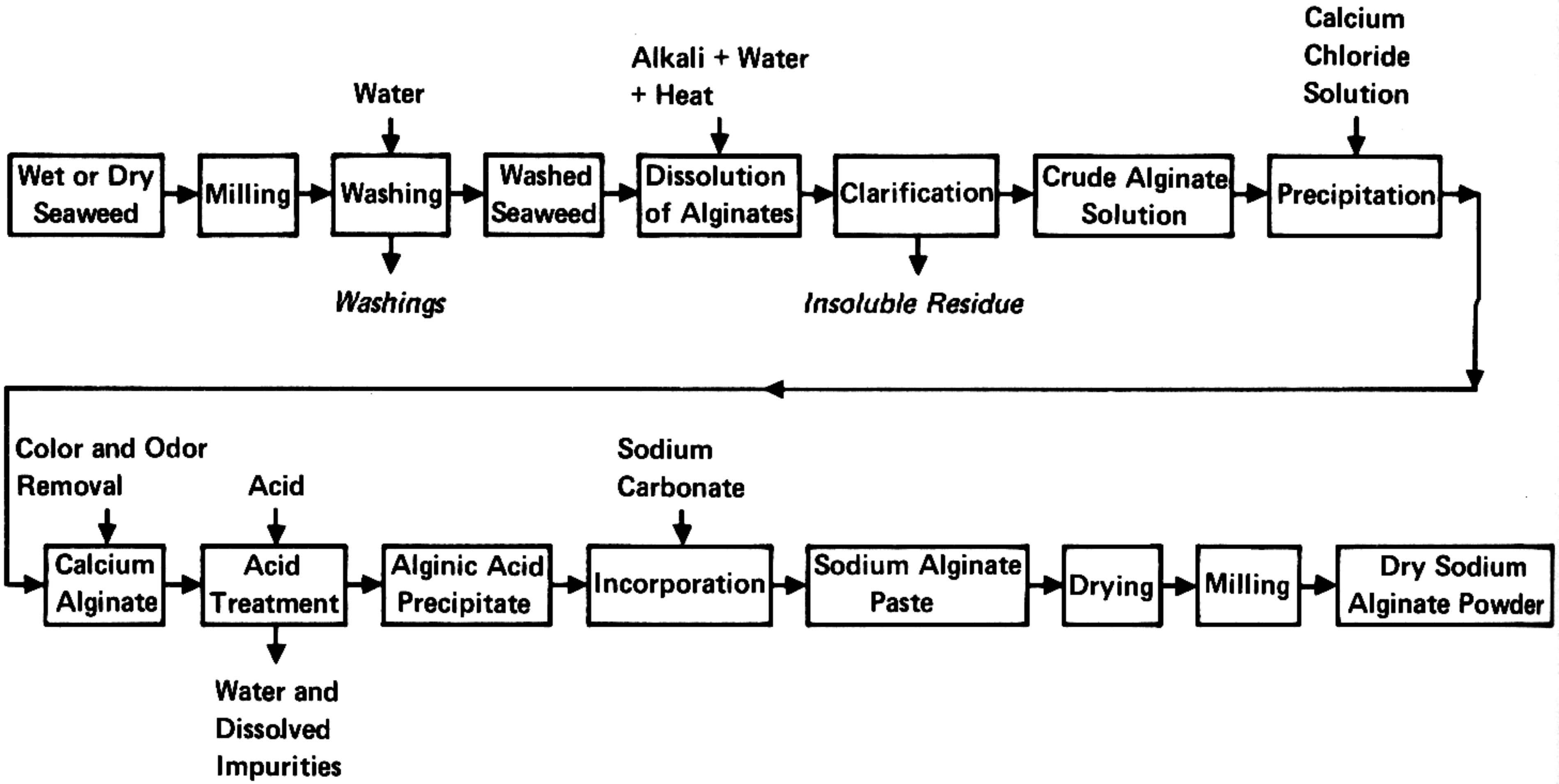


FIG. 2 Sodium alginate manufacturing process.

Source: Industrial gums handbook

A little history on agar

- **Alternating $[A(1,3)-B(1,4)]_n$ polysaccharides of marine organisms**

-

Legend has it that in about 1660, Minoya Tarozaemon, a japanese innkeeper, threw some surplus seaweed jelly into the winter night expecting it to thaw in the morning sun and to dissappear into the soil. He found, however, after several days of alternate freezing and thawing, a porous mass that could be reboiled in water and cooled to yield a gel equal to the original. He had discovered agar.

At Shimizu-mura, Japan, a monument commemorates the first commercial manufacture of agar by a relative of Tarozaemon, Miyta Hanbei of AzaShiroyama. In 1933, John Becker established the first of a series of agar companies in San Diego, California, where production continues

Source: Industrial gums handbook

Further polysaccharides from plants and Microorganisms

- **Alternating [A(1,3)-B(1,4)]$_n$ polysaccharides of marine organisms**

Agar agar: Occurs in red algae (Rhodophyceae). Used in Japan for food since the 17th century. Used in the pharmaceutical and cosmetic industry for coating of tablets and for creams and ointments; used for culture of bacteria, for electrophoresis, immunodiffusion. Agar is non-nutritive and used as an appetite blocker for dietary purposes.

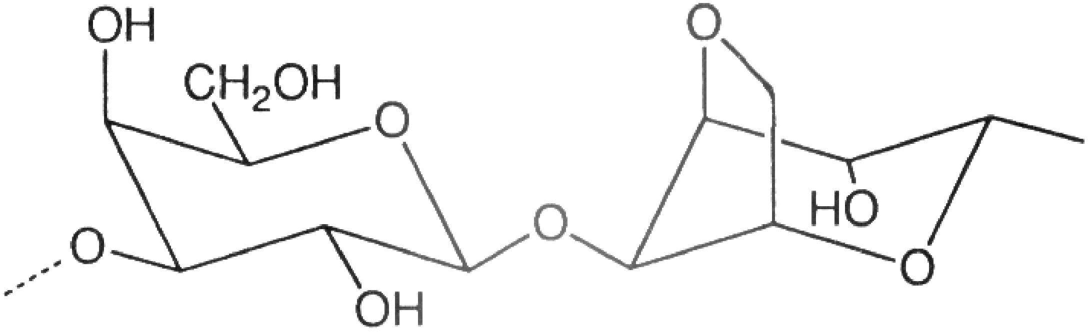


FIG. 14 An idealized repeating unit structure of agarose (agaran).

Agar Agar

Mixture of two polysaccharides:

Agarose: β-1,3-D-Galp-α-1,4-(3,6-anhydro)-L-Galp (every tenth D-Gal is sulfated at C(6)-OH). Linear chains, Mw 110 000 - 160 000. Soluble in boiling water, gives strongly acidic solutions. Agarose is a strong gel building polysaccharide: 0.2% in water forms stable gels. Metal ions are required for gel formation.

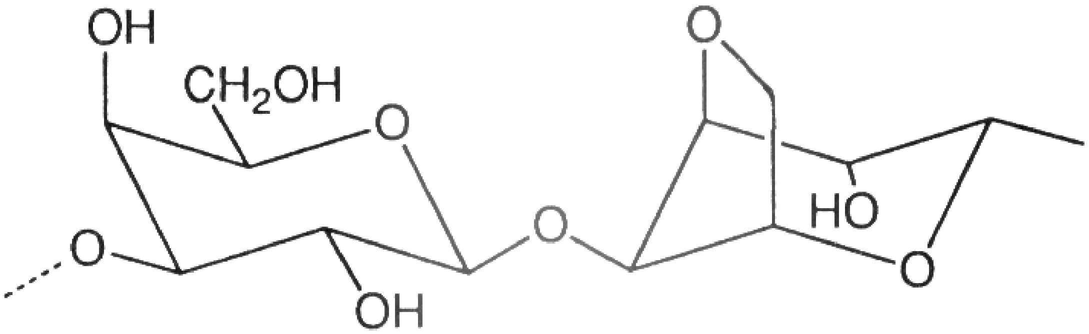
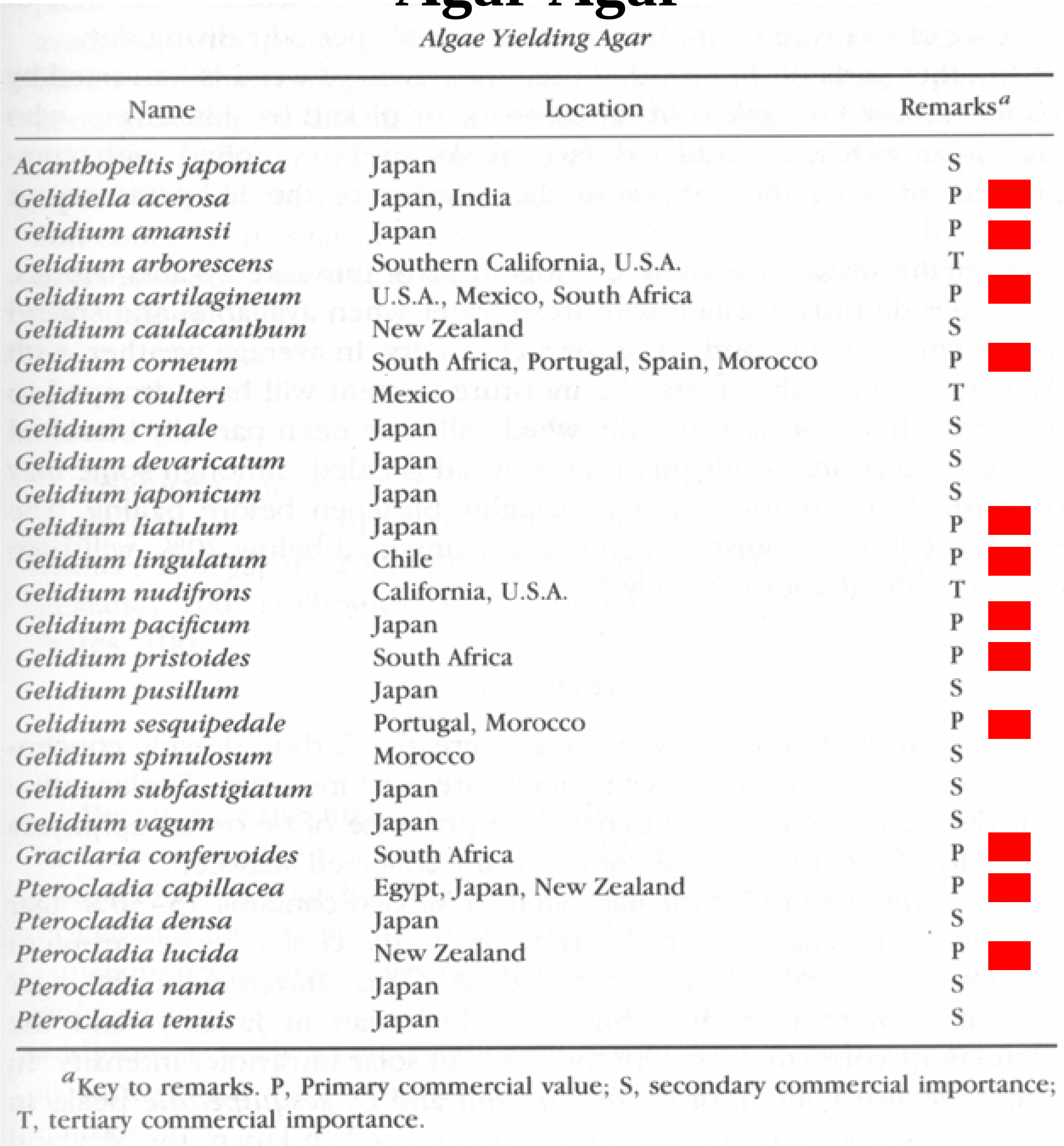


FIG. 14 An idealized repeating unit structure of agarose (agaran).

Agar Agar



Algae Yielding Agar

Name	Location	Remarks[a]	
Acanthopeltis japonica	Japan	S	
Gelidiella acerosa	Japan, India	P	🟥
Gelidium amansii	Japan	P	🟥
Gelidium arborescens	Southern California, U.S.A.	T	
Gelidium cartilagineum	U.S.A., Mexico, South Africa	P	🟥
Gelidium caulacanthum	New Zealand	S	
Gelidium corneum	South Africa, Portugal, Spain, Morocco	P	🟥
Gelidium coulteri	Mexico	T	
Gelidium crinale	Japan	S	
Gelidium devaricatum	Japan	S	
Gelidium japonicum	Japan	S	
Gelidium liatulum	Japan	P	🟥
Gelidium lingulatum	Chile	P	🟥
Gelidium nudifrons	California, U.S.A.	T	
Gelidium pacificum	Japan	P	🟥
Gelidium pristoides	South Africa	P	🟥
Gelidium pusillum	Japan	S	
Gelidium sesquipedale	Portugal, Morocco	P	🟥
Gelidium spinulosum	Morocco	S	
Gelidium subfastigiatum	Japan	S	
Gelidium vagum	Japan	S	
Gracilaria confervoides	South Africa	P	🟥
Pterocladia capillacea	Egypt, Japan, New Zealand	P	🟥
Pterocladia densa	Japan	S	
Pterocladia lucida	New Zealand	P	🟥
Pterocladia nana	Japan	S	
Pterocladia tenuis	Japan	S	

---

[a] Key to remarks. P, Primary commercial value; S, secondary commercial importance; T, tertiary commercial importance.

Further polysaccharides from plants and Microorganisms

Alternating $[A(1,3)\text{-}B(1,4)]_n$ polysaccharides of marine organisms

Carrageenan: Occurs in North Atlantic red algae. Also named Irish moss. The name is from the Irish city Carragheen.

Several types are known: most important κ-carrageenan (kappa-c.): β-D-Galp-4-sulfate-α-D-(3,6-anhydro)-Galp; DP ca. 1200. Used for similar applications like agar agar; for flocculation of proteins.

Many carrageenans form thermoreversible gels depending on the Hoffmeister ion series depending on charge screening and the associated stabilization of the double helix ($K^+ > Na^+ > Li^+$)

Different Carrageenans

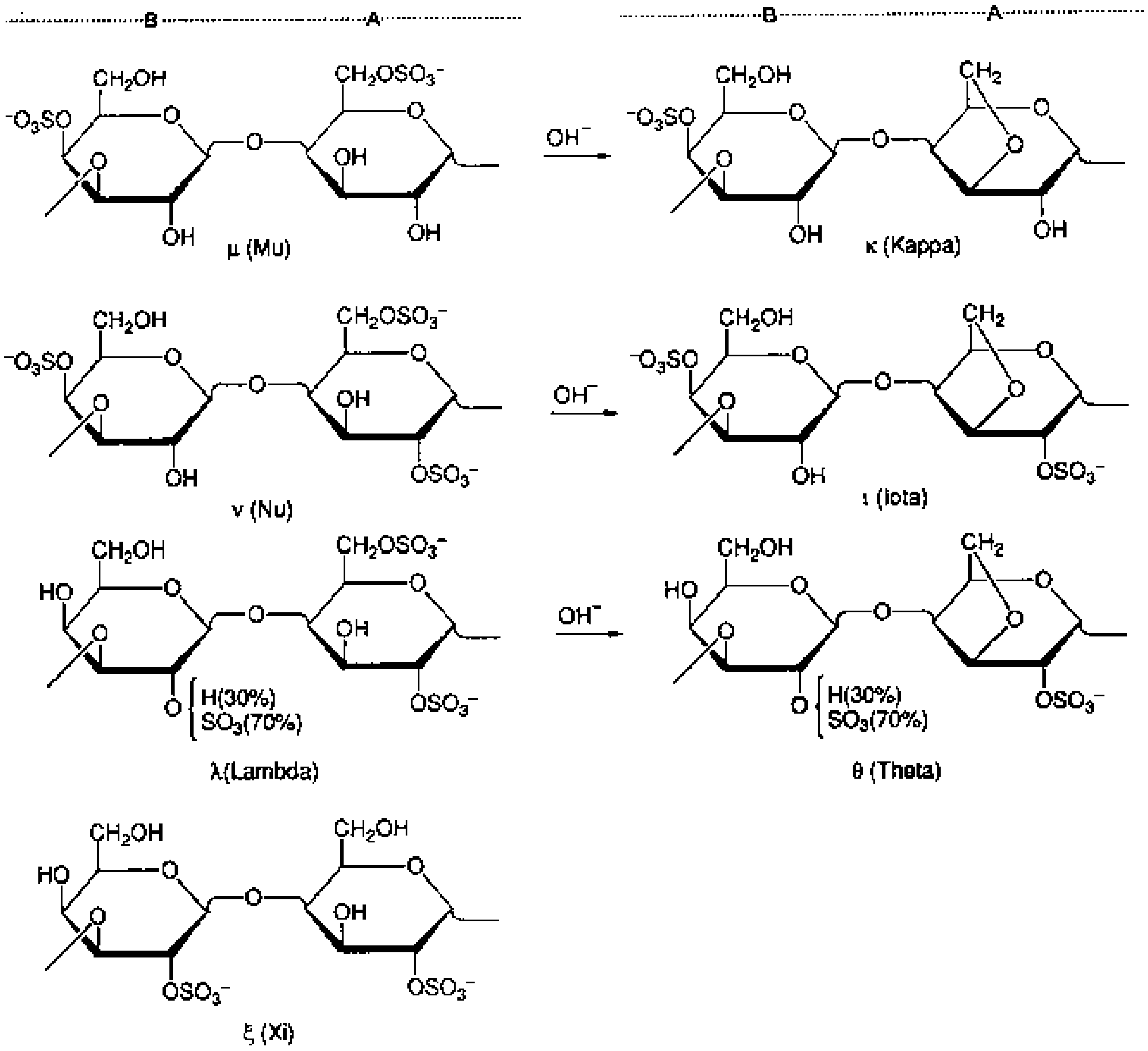


FIG. 3 Structures of basic carrageenan repeating units.

Different Carrageenans

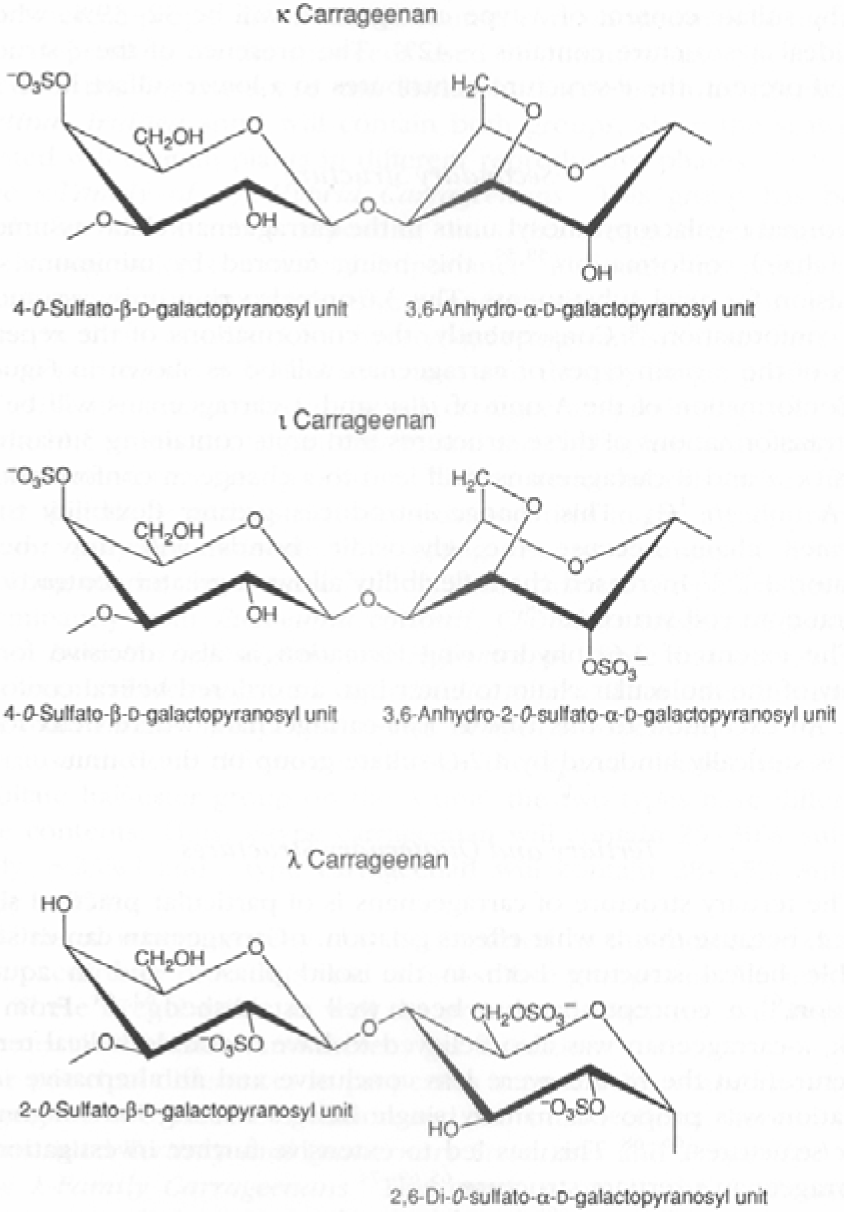


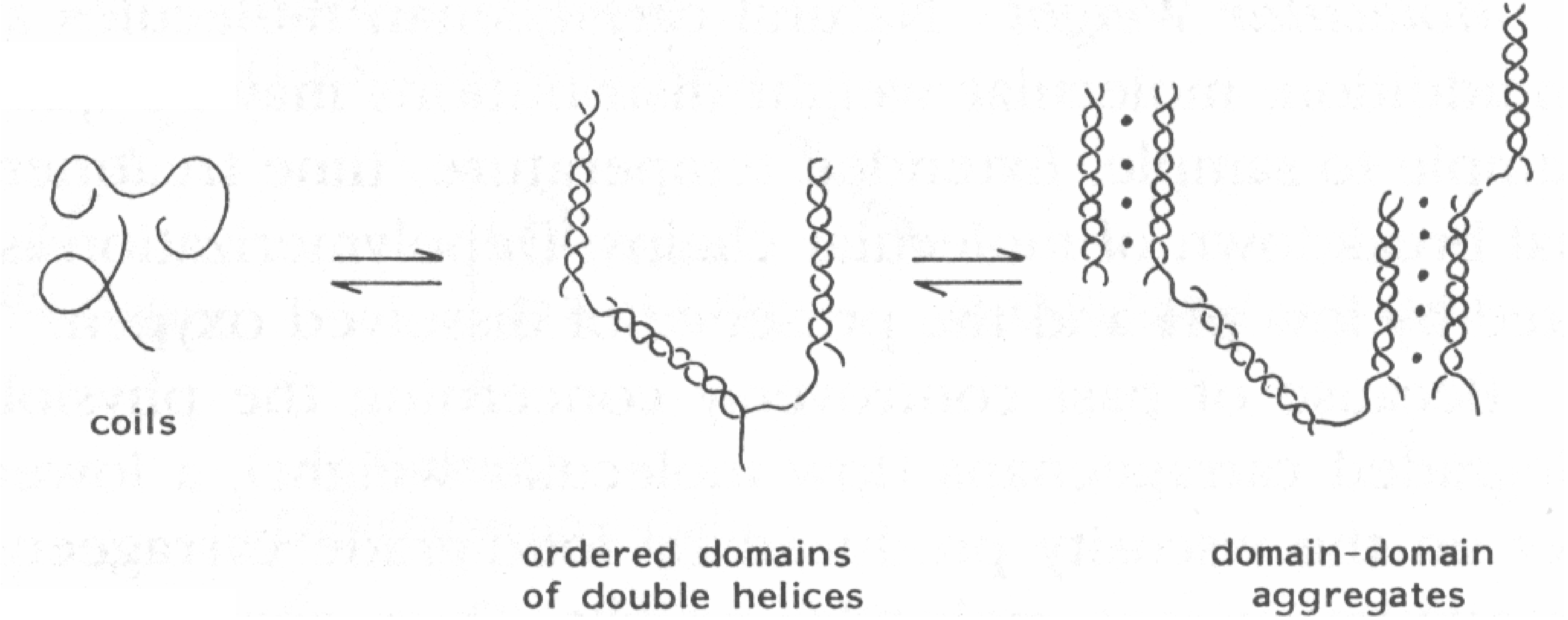
FIG. 4 Stereochemical representation of basic carrageenan repeating units. The B-units of all three basic types and the A-unit of λ-carrageenan assume the $^4C_1$ conformation, whereas the A-units of κ- and ι-carrageenan assume the $^1C_4$ conformation.

Gelling, double helix formation

Gelling, double helix formation

Non gelling

κ-Carrageenan gelling mechanism



Domain model for κ-carrageenan gelation

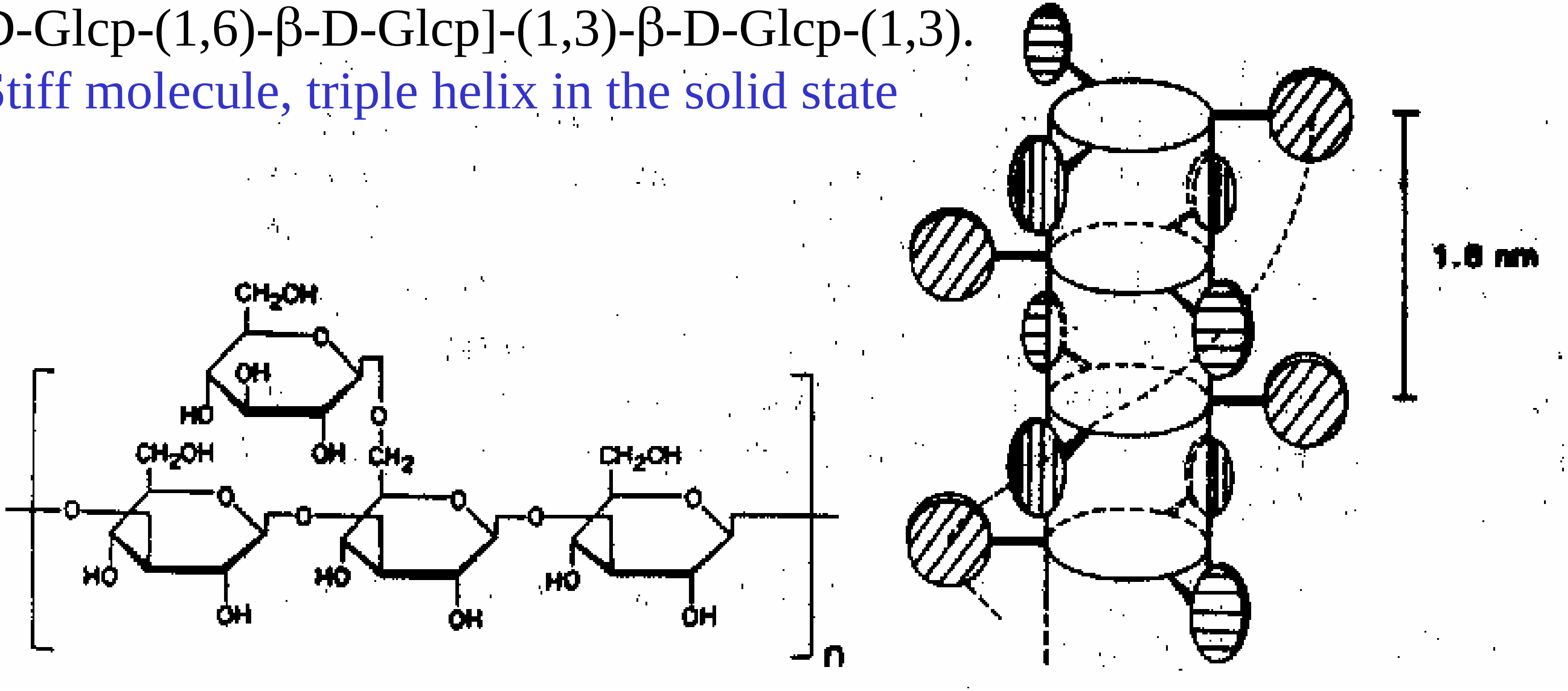
Further polysaccharides from plants and Microorganisms

- **Microbial (exo)polysaccharides**

Gellan: from *Pseudomonas elodea*. -3)-β-D-Glcp-(1,4)-β-D-GlcpA-(1,4)-β-D-Glcp-(1,4)-α-L-Rhap(1-. Gel formation in the presence of Na, K, Mg, Ca ions.

Schizophyllan: from *Schizophyllum commune*. -3)-β-D-Glcp-(1,3)-[β-D-Glcp-(1,6)-β-D-Glcp]-(1,3)-β-D-Glcp-(1,3).

Stiff molecule, triple helix in the solid state



Structure of Schizophyllan

Ebert p369

Further polysaccharides from plants and Microorganisms

Microbial (exo)polysaccharides

Emulsan: from *Acinetobacter calcoaceticus* RAG-1. Capsular polysaccharide, composed of GalN, GalAN, $GlcN_2$. Contains long chain fatty acids as esters and amides. Technical product, made by fermentation, used for emulgation of mineral oils in water.

Pullulan: from fungi *Aureobasidium pullulans*. Linear Polysaccharide consisting of α-1,6-linked maltotriose units.

Xanthan: from *Xanthomonas campestris* NRRL B-1459. Branched, acidic heteropolysaccharide, Mw ca. $3\text{-}7 \bullet 10^6$. The backbone is cellulose. Statistically, every second Glc unit contains a branch of β-D-Man-(1,4)-β-D-GlcA-(1,2)-α-D-Man-(1,3→); 50% of the terminal Man units are acetals of pyruvic acid.

Xanthan forms extremely viscous solutions.

Xanthan

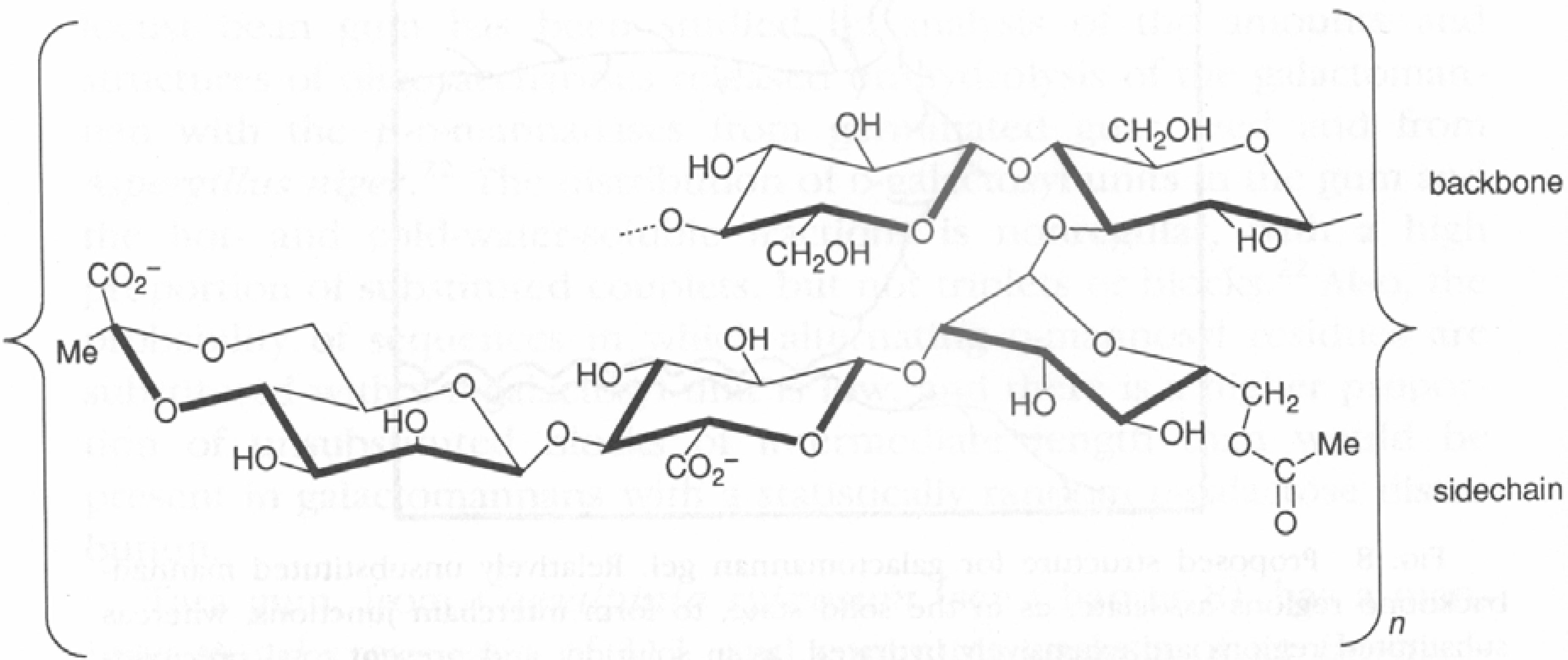


FIG. 9 Xanthan.

Primary Structure of Xanthan

Ebert p371

Xanthan

Xanthan solution structure investigation suggested a rod-like or worm-like conformation with low degree of flexibility

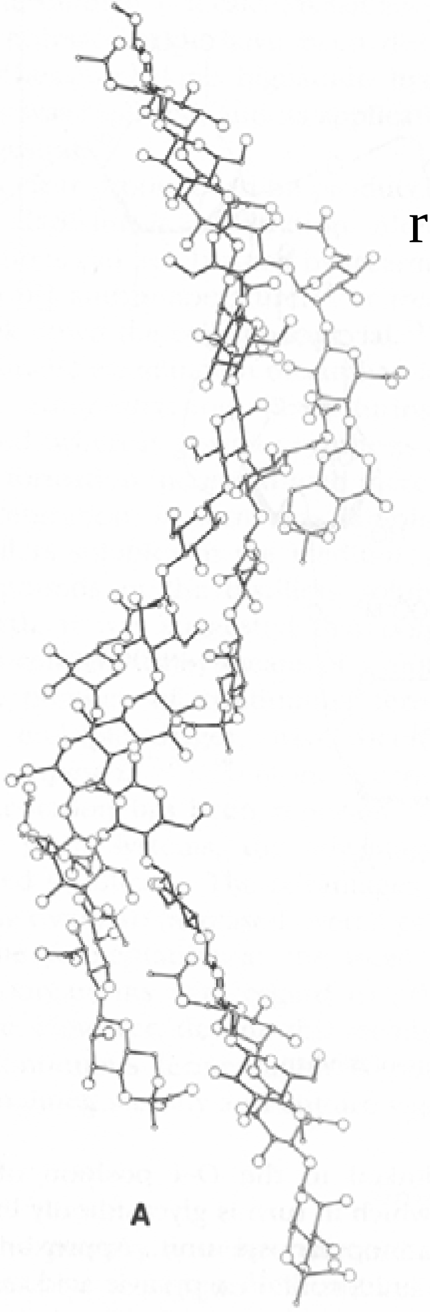
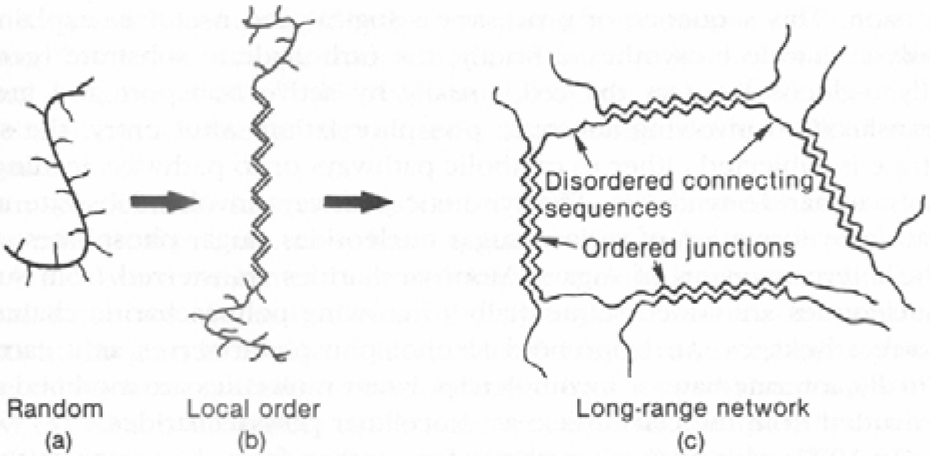


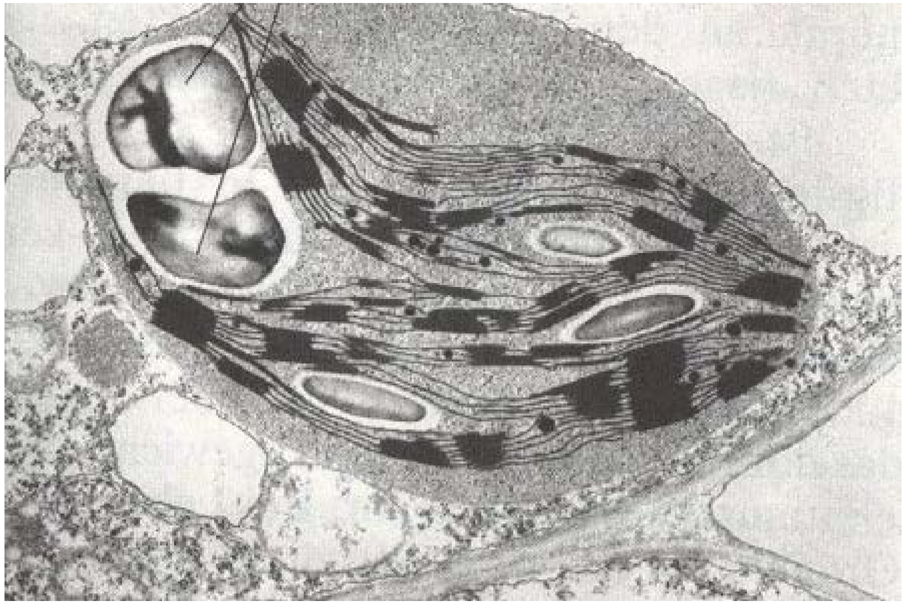
FIG. 2 Molecular conformation of xanthan as determined by modeling.[45] (A) Vie perpendicular to helix axis. (B) View down helix axis.

Helix formation



2.6. Starch

- Starch is the most important storage saccharide in plant cells
- Occurs as large Aggregates or Granula (Energy storage)
- Strong hydration of starch molecules due to external hydroxyl groups which undergo hydrogen bonding with water.



Starch granula as photosynthesis product in a Chloroplast

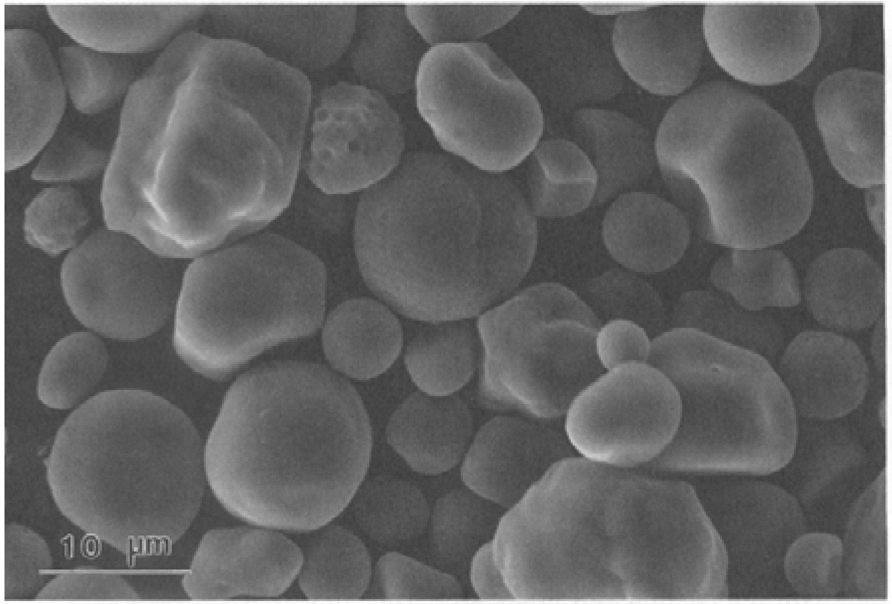


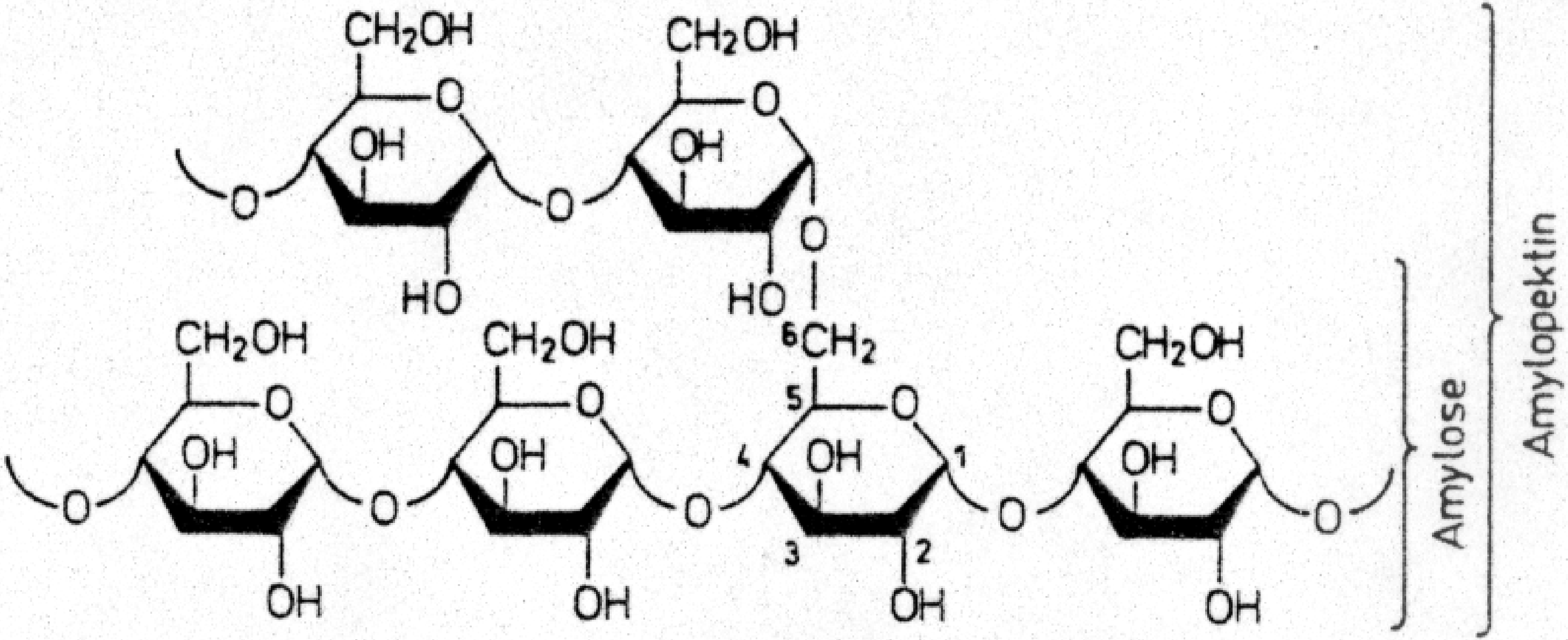
FIG. 1 Photomicrograph of yellow dent corn starch.

Corn starch

2.6. Starch

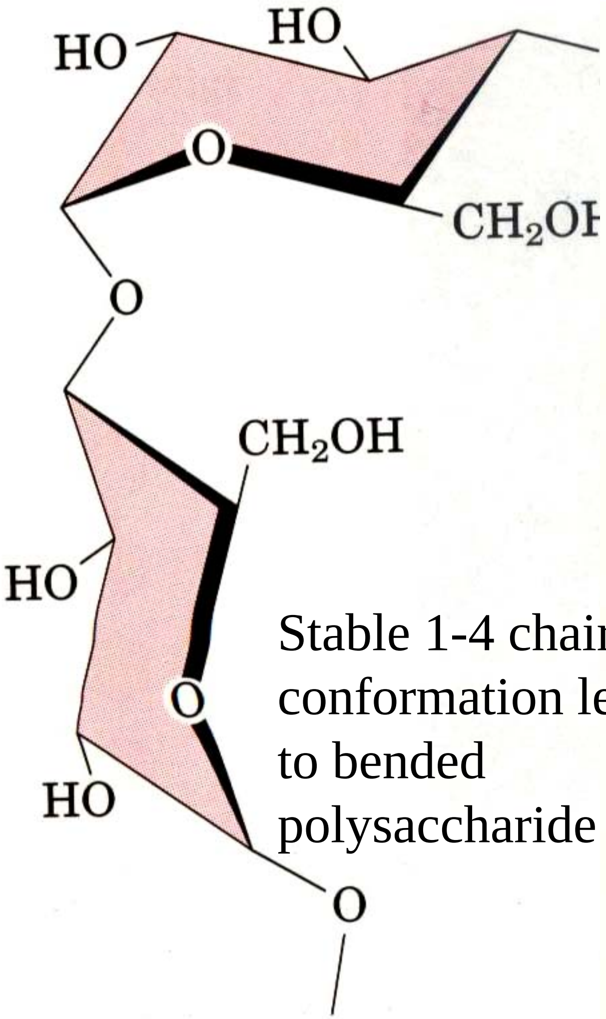
Starch is a mixture of amylose [poly(1,4-α-D-glucose)] and amylopectin [1,6-branched amylose].

Amylose 5 – 500 kDa linear
Amylopectin up to 1 Mda branched



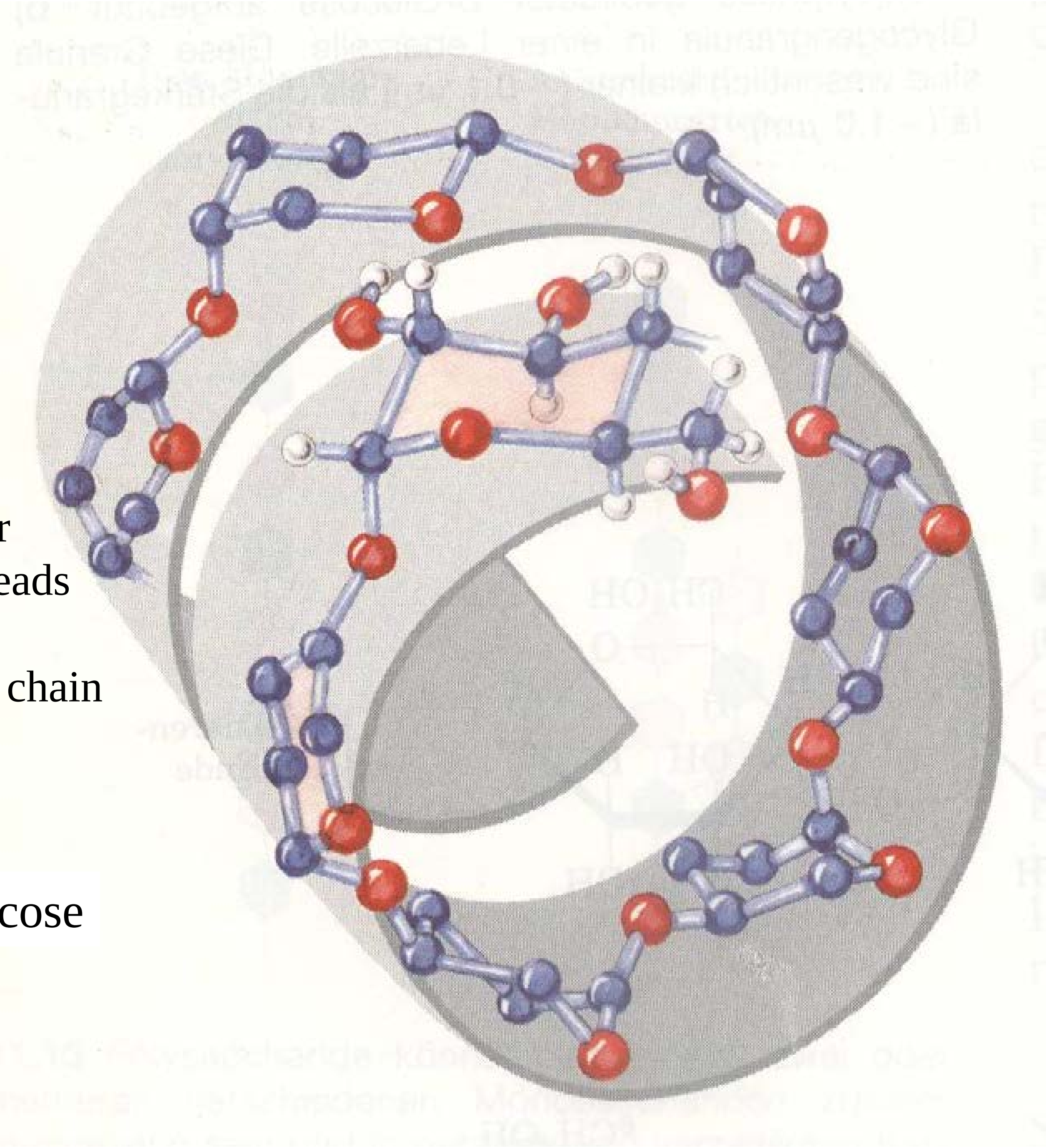
2.6. Starch

- Large production: 1976 worldwide 8 x10^6 tons
- Degradation to glucose by acids or enzymes (glucosidases) finally to sugar
- The most important human nutrition substance (a grown up human needs ca. 500 g carbohydrates, mainly in form of starch)
- 1 g starch yields 17 kJ (4kcal) energy
- Large amounts of starch are further processed to alcohols
- Also application as adhesive and as thickener in the food industry
- Stiffening of textiles was already invented in 1525 in Flandern where the heat transforms starch to dextrins
- Nowadays important role as renewable source in Biotechnology for production of yeast, glucose, isomerose, sorbit, pullulan, ethanol etc.

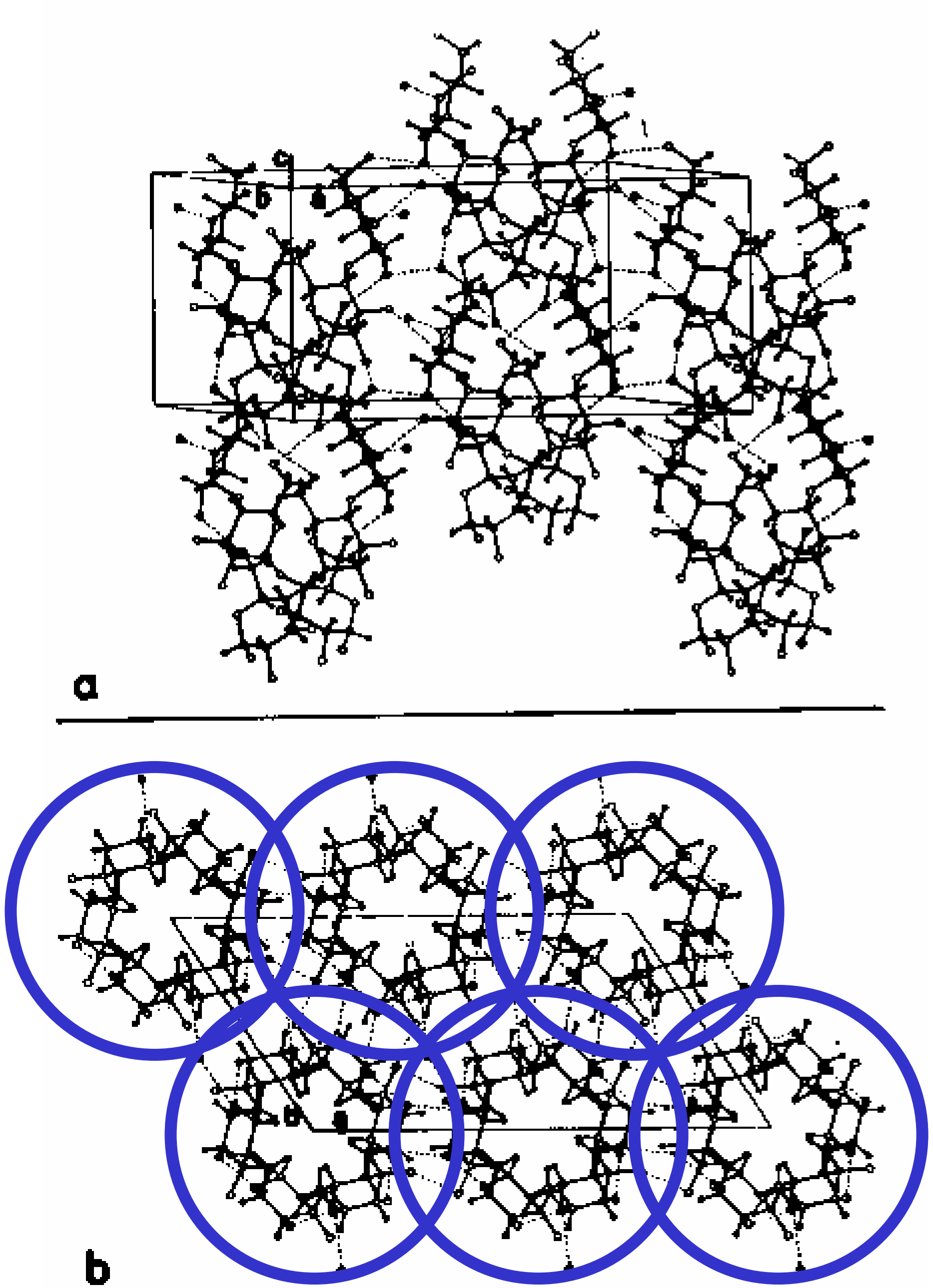


Stable 1-4 chair conformation leads to bended polysaccharide chain

(α1→4)-connected D-Glucose

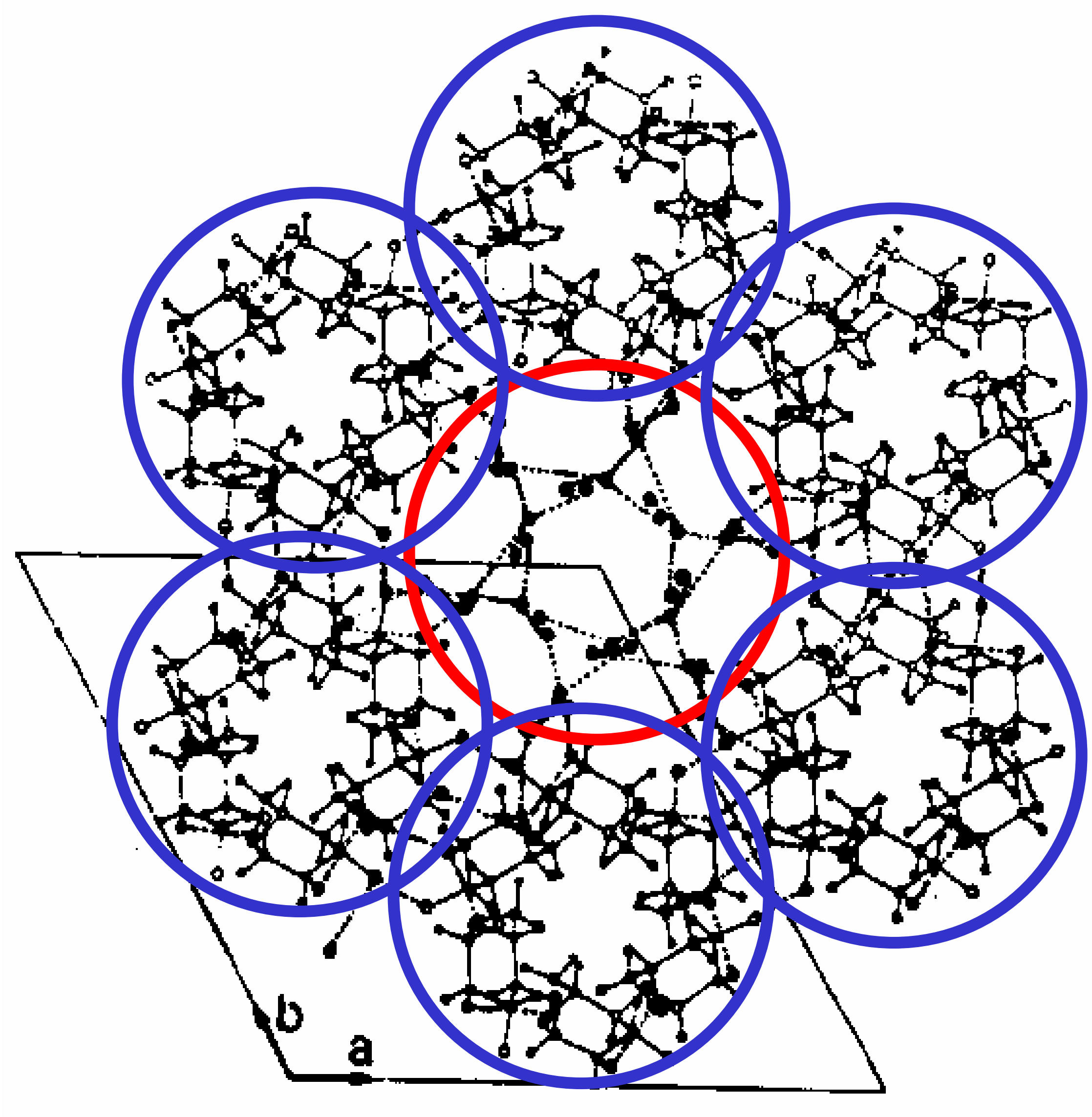


Amylose



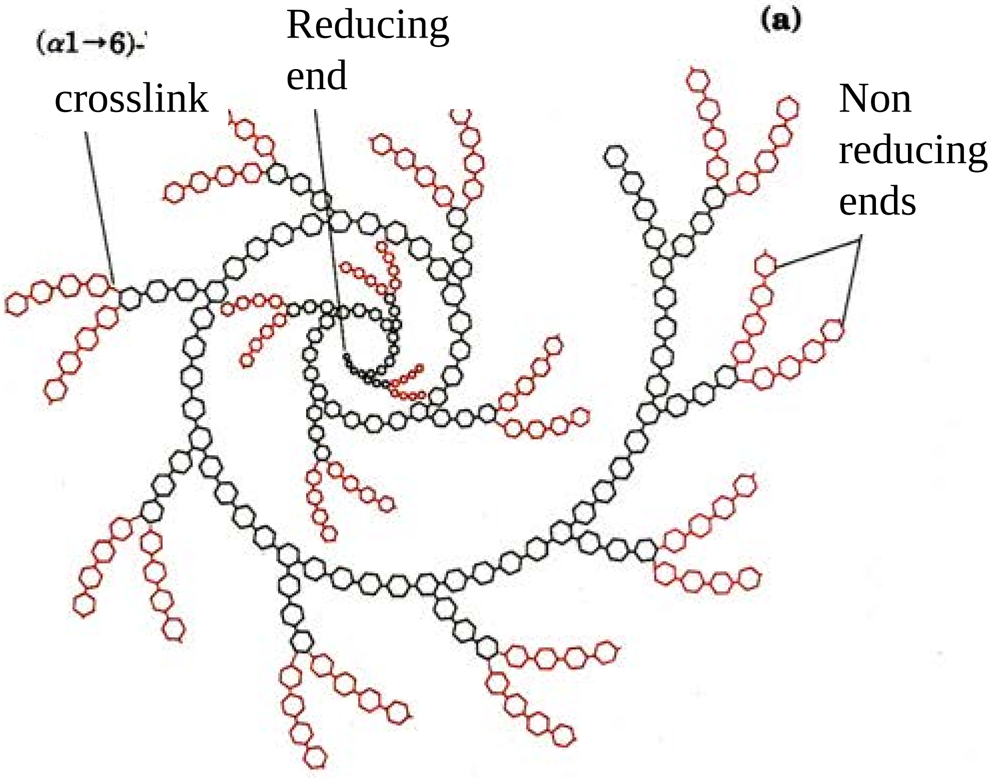
Elementary cell and double helix in amylose (tertiary structure)

Ebert p411

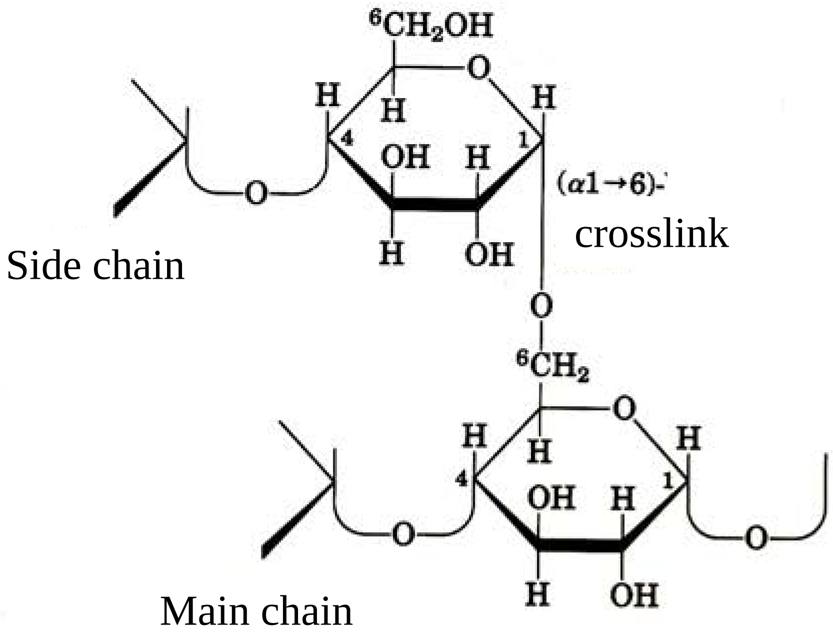


Amylose-B-structure, composed of six double helices. Note the water molecules filling the center.

Ebert p412



(b)

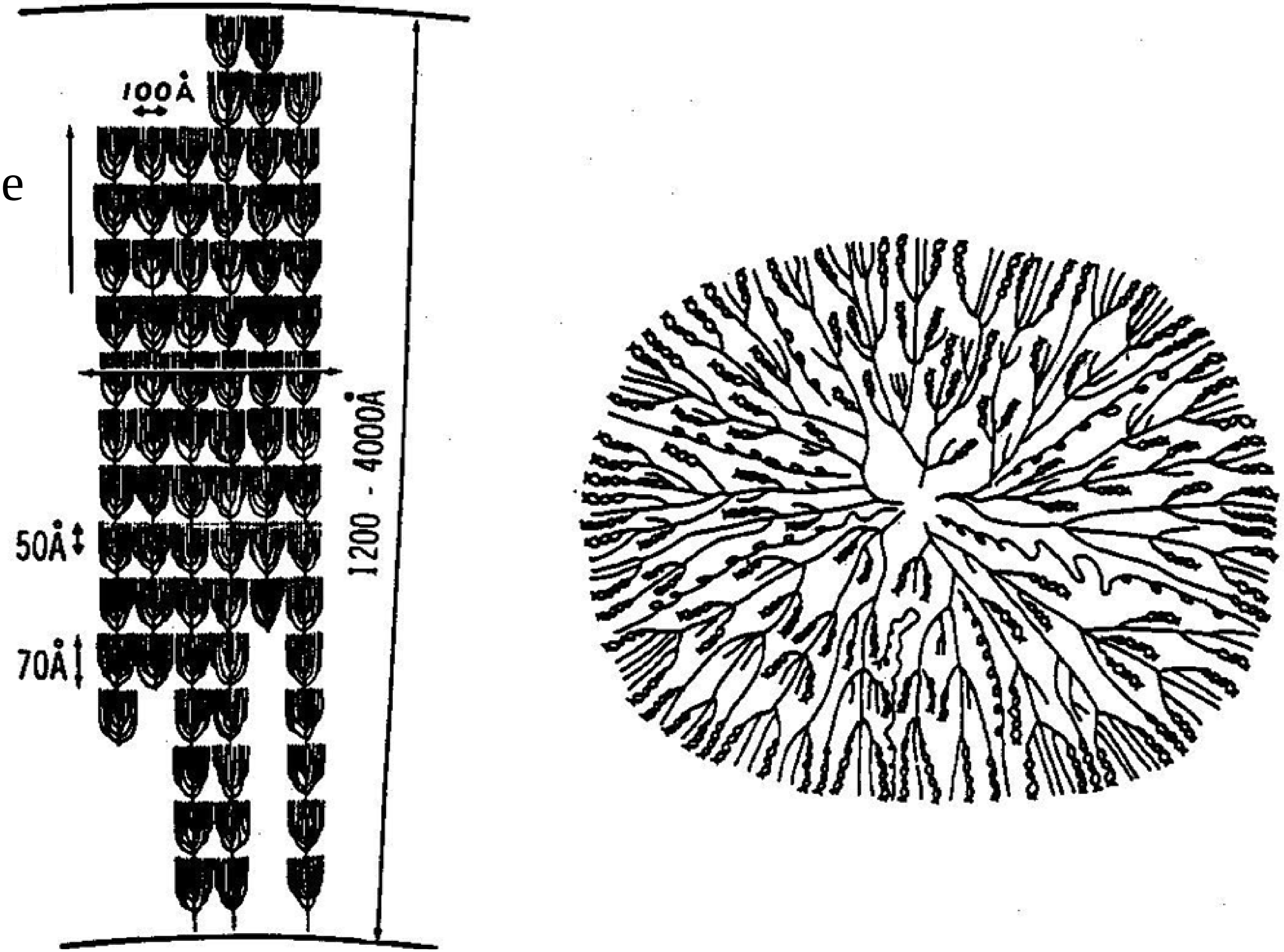


(c)

Every red hexagon represents an external glucose residue, which is stepwise enzymatically cleaved upon the intrazellular mobilisation of starch for energy production

Amylopectin

Schematic view of amylopectin



Growth ring

Molecule growth

Crystal growth

Growth ring

Schematic view of the molecular orientation of amylopectin in the growth layers of a strach grain

Ebert p413

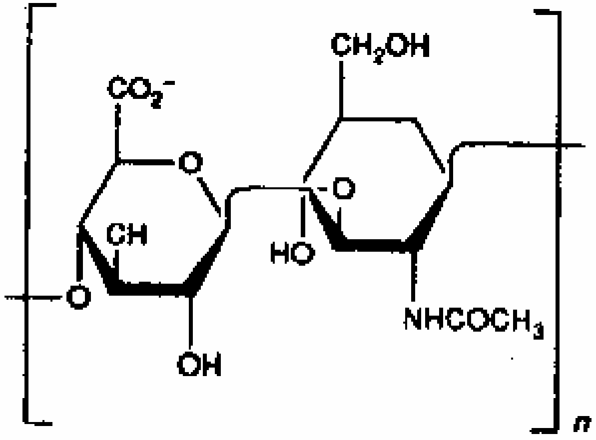
2.7. Other important polysaccharides

Mucopolysaccharides

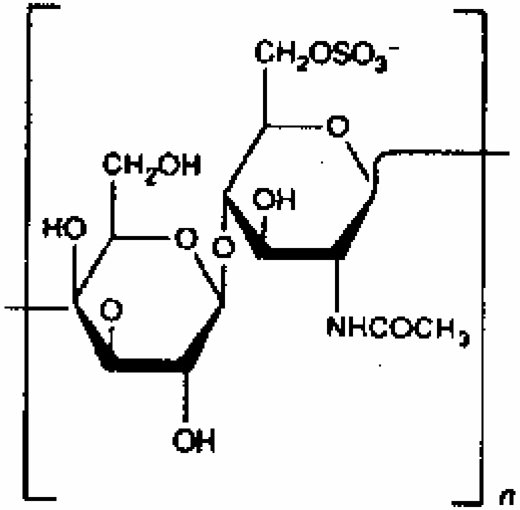
Main components of connective tissues in animals. General type: $[A(1,3)\text{-}B(1,4)]_n$

	A	B
Hyaluronic acid	β-D-GlcA	β-D-GlcNAc
Chondroitin-4-sulfate	β-D-GlcA	β-D-Gal-4-*O*-sulfate
Chondroitin-6-sulfate	β-D-GlcA	β-D-Gal-6-*O*-sulfate
Dermatansulfate	α-D-IdoA and β-D-GlcA	β-D-Gal-4-*O*-sulfate / 6-*O*-sulfate
Keratansulfate	β-D-GlcNAc and -6-*O*-sulfate	β-D-Gal and -6-*O*-sulfate

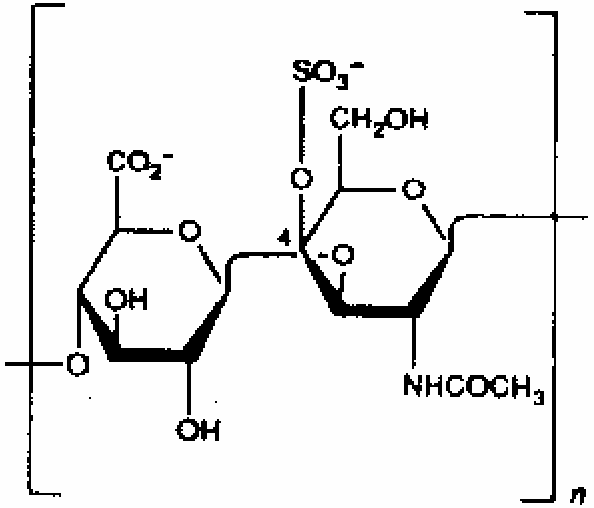
2.7. Other important polysaccharides



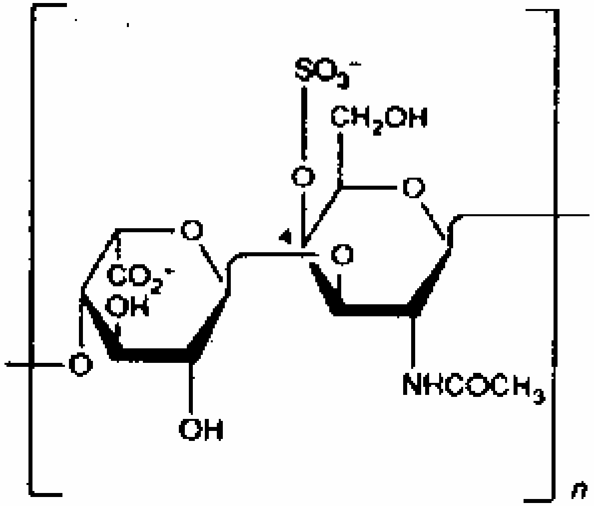
Hyaluronan
(HA)



Keratan sulfat
(KS)



Chondroitin 4-sulfat
(CS-4S)



Dermatan sulfat
(DS)

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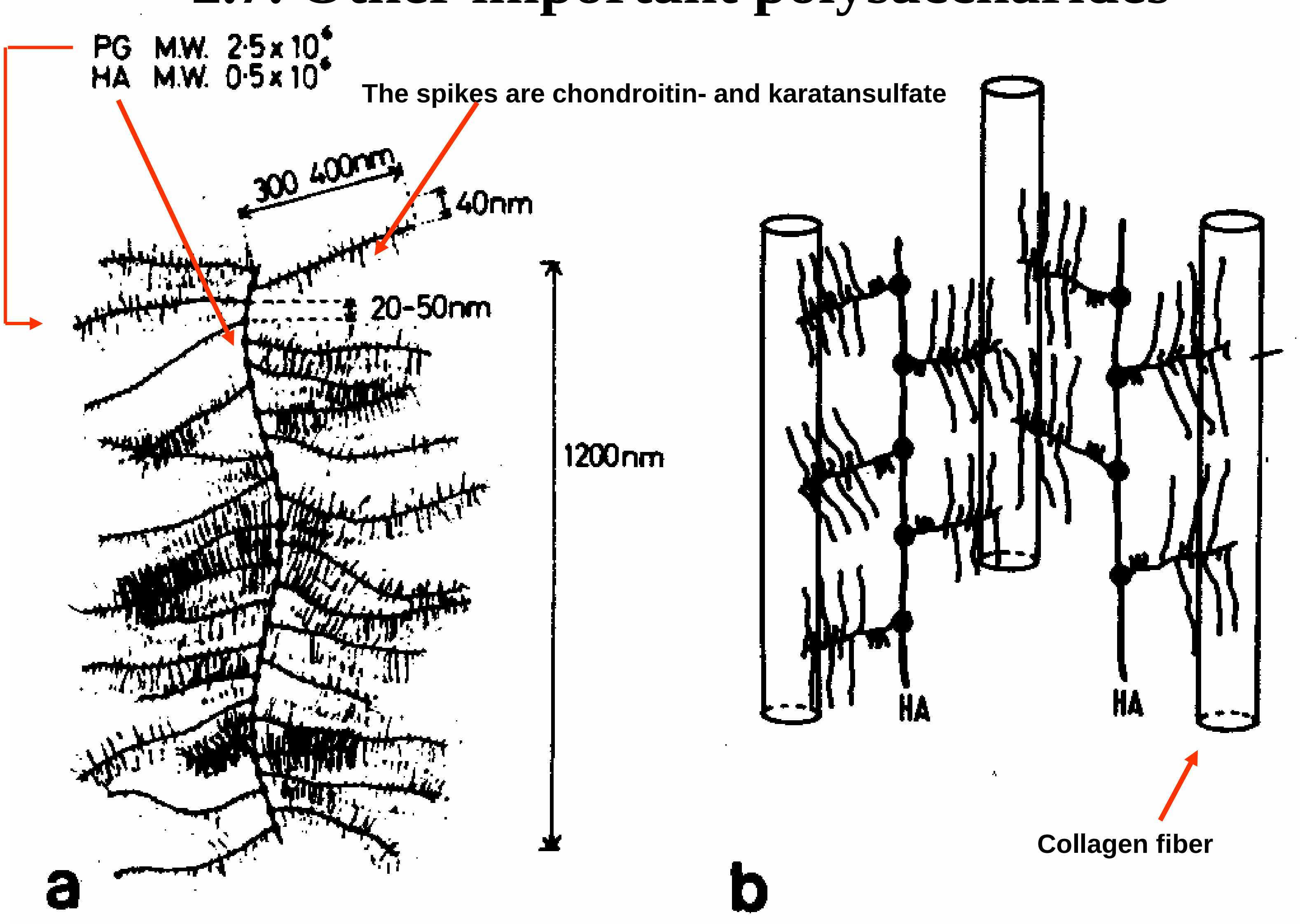
Mucopolysaccharides

2.7. Other important polysaccharides



The spikes are chondroitin- and karatansulfate

Collagen fiber

Schematic view of a proteoglycan-hyaluronic acid complex

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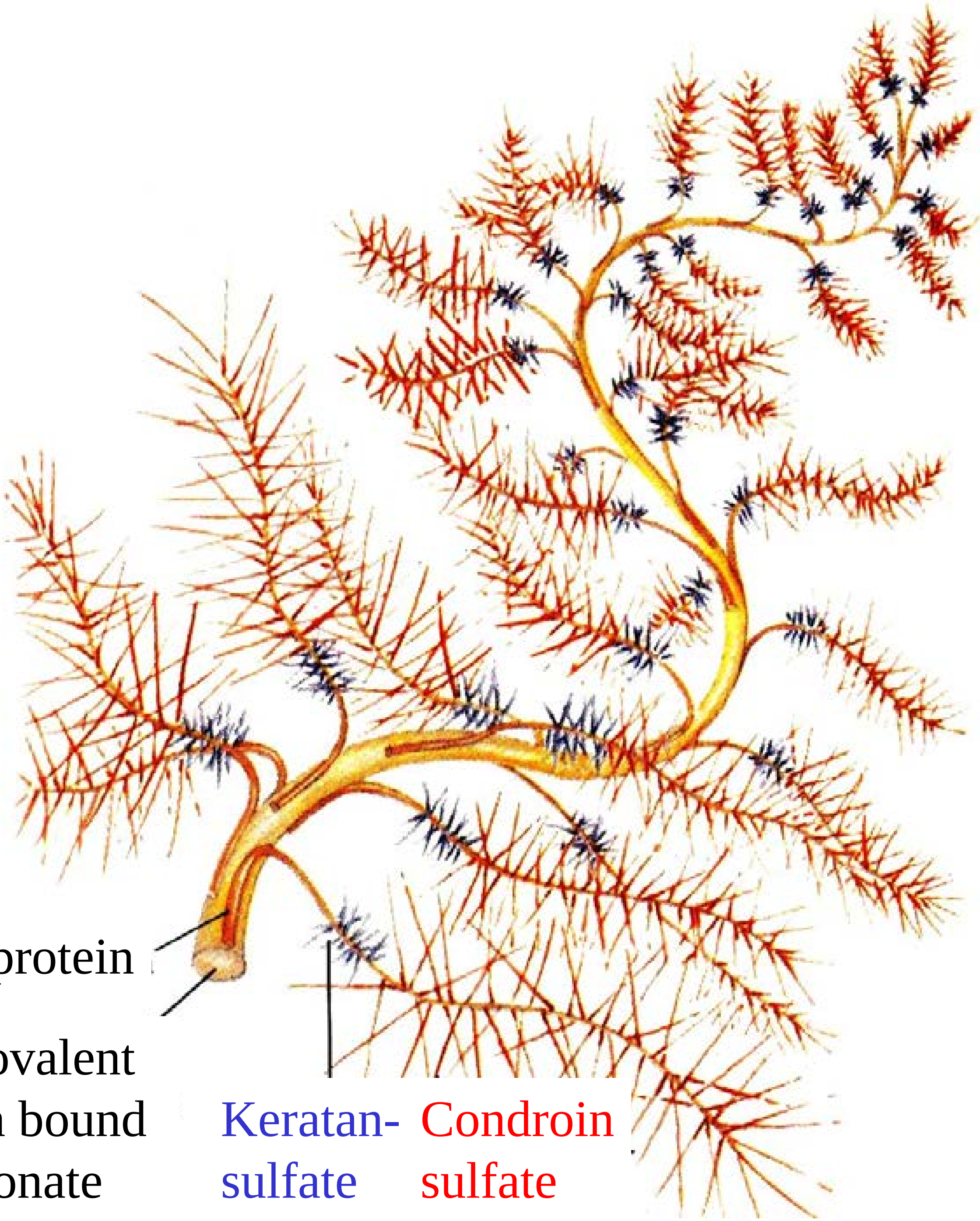

Core protein

Non covalent protein bound hyaluronate

Keratan-sulfate

Condroin sulfate

2.7. Other important polysaccharides

<u>Heparansulfate</u> and <u>heparin</u>

Heparansulfate is a polysaccharide of the type [A(1,4)-B(1,4)]$_n$ where
A = α-D-GlcN-6-*O*-sulfonate (some residues are *N*-acetylated or *N*-sulfonated),
B = β-D-GlcA or α-L-IdoA-2-*O*-sulfonate

Thus, the repeating unit in heparansulfate is a tetrasaccharide: [-4)-α-L-IdoA-2-*O*-sulfate-(1,4)-α-D-GlcN-*N*-sulfonate-6-*O*-sulfonate-(1,4)-β-D-GlcA-(1,4)-β-D-GlcNAc-6-*O*-sulfonate-(1-]. Heparansulfate is a component of Proteoheparansulfate which is a core protein containing four heparansulfate side chains. Proteoheparansulfate is a main component of the glycocalix lining of blood vessels. It prevents blood clotting.

Heparin (Mw 17000 - 20000) is similar to heparansulfate, containing tetrasaccharide units of β-1,4-linked D-GlcN-*N*,6-*O*-disulfonate and D-GlcA-2-(or 3-)-*O*-sulfonate.
Heparin is a potent anticoagulant which is used e.g. in surgery to prevent thromboses.

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