

1. PLANT CARBOHYDRATE MACROMOLECULES

1.0 Introduction: Overview of Plant Polysaccharides

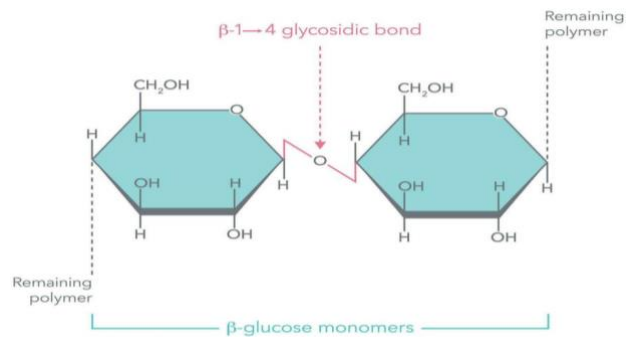
Plants dedicate a significant portion of their fixed carbon to synthesising polysaccharides for structural integrity and energy storage. Listed below are the major plant polysaccharides in approximate order of biological indispensability.

1. **Cellulose** – The most abundant biopolymer on Earth; the primary load-bearing scaffold of every plant cell wall.
2. **Pectin** – The major component of the middle lamella; acts as intercellular cement and regulates wall porosity.
3. **Starch** – The universal carbon and energy reserve of plants.
4. **Hemicellulose** – Matrix polysaccharides (e.g., xyloglucan) that hydrogen-bond to cellulose microfibrils.
5. **Inulin** – A fructan storage reserve crucial for osmoregulation, with prebiotic and pharmaceutical relevance.
6. **Galactomannan** – Legume endosperm storage polysaccharides (e.g., guar gum) serving as energy and water-retaining matrix.
7. **Glucomannan** – A hemicellulosic component and storage polymer in tubers (e.g., konjac).
8. **Arabinogalactan** – Structural glycoproteins and wound-sealing gum exudates (e.g., gum arabic).

1.1 Cellulose

Structure

Cellulose is the most abundant extracellular structural, unbranched homopolymer of D-glucose linked by **β -(1→4) glycosidic bonds**. To form this bond, every alternate glucose molecule is inverted or rotated by 180 degrees, creating a straight, rigid, and flat ribbon-like chain. The flat, ribbon-like conformation allows extensive inter- and intra-chain hydrogen bonding, forming highly crystalline microfibrils.



It is an intricate polysaccharide that constitutes the fundamental building block of the cell walls of plants. A cellobiose molecule of 1.3 nm in length can be regarded as the recurring basic unit of cellulose.

Biochemical Functions

Cellulose serves many functions in living organisms.

1. **Provides Rigidity to Plants:** Cellulose primarily offers structural support to plant cells. It forms a robust and rigid cell wall, giving plant cells their characteristic shape and preventing them from collapsing under pressure. Microfibrils provide tensile strength and are embedded in a matrix of hemicelluloses, pectins, and structural proteins.
2. **Regulates Water Uptake and Transportation:** It also helps regulate water uptake in plants and transportation. It helps maintain the osmotic pressure in plant cells, ensuring proper water balance and turgidity.
3. **Provides Growth and Development:** Cellulose is involved in cell growth and expansion during plant development. As the plant grows, new cellulose microfibrils develop, increasing the size of the cell wall and enabling cell elongation.
4. **Protects from Pathogens:** The cellulose cell wall is a physical barrier, protecting plant cells from external threats, such as pathogens and herbivores.
5. **Produces Biofilms:** Some bacteria secrete cellulose to form biofilms that help microorganisms to attach and organize into colonies.
6. **Dietary fibre:** promotes peristalsis and faecal bulking in human nutrition.

Although cellulose are indigestible by humans due to the absence of β -1,4-glucosidases (cellulases). Ruminants and termites rely on symbiotic microbiota that secrete cellulase systems (endoglucanases, cellobiohydrolases, β -glucosidases).

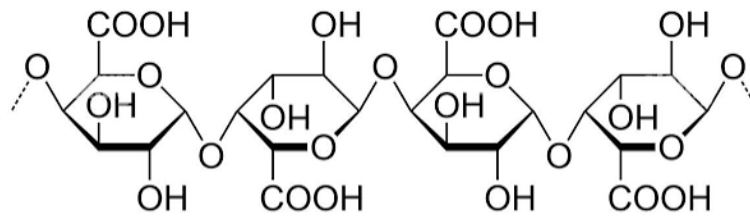
1.2 Pectin

Pectin is a structurally complex heteropolysaccharide found in plant cell walls, composed primarily of a linear chain of α -(1,4)-linked D-galacturonic acid. The structure is heterogeneous, consisting of alternating "smooth" and "hairy" regions made up of four major domains:

- a. **Homogalacturonan (HG):** The "smooth" region. It is a long, linear chain of galacturonic acid units that can be partially **methyl-esterified** (at the carboxyl group) and **acetyl-esterified**. The degree of methylation determines how pectin behaves, such as how it forms gels in jams and jellies.
- b. **Rhamnogalacturonan I (RG-I):** The "hairy" region. It consists of a repeating disaccharide backbone of galacturonic acid and rhamnose. Long, neutral sugar side chains (primarily **arabinan** and **galactan**) branch off from the rhamnose residues.
- c. **Rhamnogalacturonan II (RG-II):** A highly complex, branched domain containing various unusual sugars (like apiose). Despite its small size, it is crucial for cross-linking pectin chains.

- d. **Xylogalacturonan (XGA):** A variant of the HG backbone that is substituted with xylose side chains.

Together, these domains form a porous, gel-like network that provides structure and acts as a "glue" between plant cells.



Biochemical Functions

1. In Plants

- Cell Adhesion & Strength:** Pectin acts as the primary "glue" (the middle lamella) that binds adjacent cell walls together, providing mechanical rigidity and flexibility.
- Water Regulation:** It helps control cell wall porosity and hydration, which is vital for regulating water movement and maintaining turgor pressure.
- Growth and Ripening:** Pectin's structure shifts to allow for cell expansion during growth. During fruit ripening, it is broken down by specific enzymes, causing the fruit to soften.

2. In Human Nutrition and Health

- Prebiotic Effect:** As a soluble fiber, pectin resists digestion in the stomach and small intestine. Once in the colon, it is fermented by gut microbiota, stimulating the growth of beneficial bacteria and producing short-chain fatty acids.
- Metabolic Support:** It helps manage blood sugar by slowing the absorption of carbohydrates. It also binds to bile acids in the gut, which can assist in lowering LDL (bad) cholesterol levels.
- Gastrointestinal Regulation:** In the digestive tract, pectin expands to form a gel. This adds bulk to stool, slows down gastric emptying, and can absorb toxins and harmful bacteria, which is why it is often used as a natural anti-diarrheal.

3. Biomedical Applications

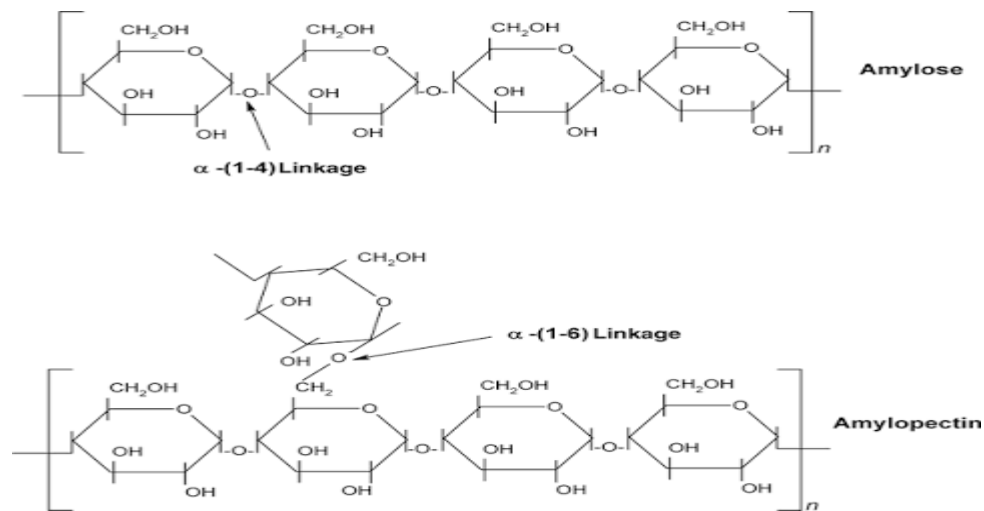
- Drug Delivery:** Pectin's gelling properties make it an ideal carrier for hydrogel-based and colon-specific drug delivery systems, allowing medication to be released at specific rates and locations within the body.
- Tissue Engineering & Wound Healing:** Because it is highly biocompatible, pectin is used in bio-composite materials to aid in wound healing and tissue regeneration.

1.3 Starch

Starch is a storage glucan composed of two polymers that work together to optimize this storage and retrieval process:

- Amylose(20–30%):** A straight-chain polymer (linear α -(1→4)-linked D-glucose chains) that coils into a tight, compact shape, allowing plants to store maximum energy in a minimal amount of space.
- Amylopectin(70–80%):** A highly branched polymer composed of a linear (1→4)-linked D-glucose backbone with α -(1→6) branch points every 24–30 residues. This creates more surface area, enabling enzymes to quickly break down the starch and release glucose on demand.

Because starch is a large, insoluble molecule, it allows plants to store vast amounts of energy without disrupting their cellular water balance (osmosis).



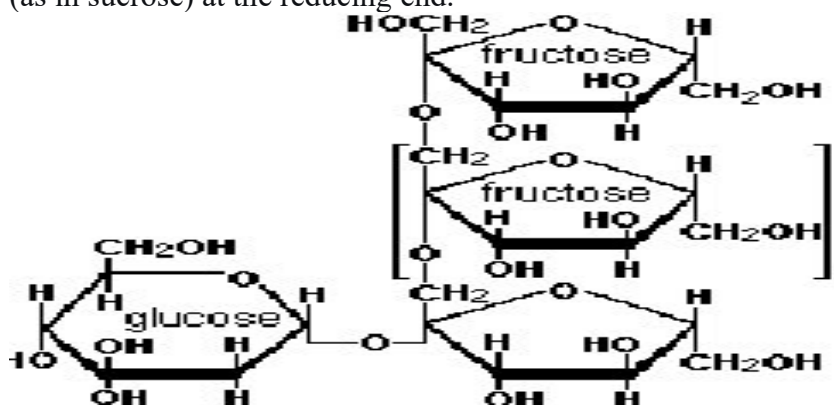
Biochemical Functions

1. **Primary energy reserve in plants:** transient starch is synthesised in chloroplasts during the day and degraded at night; storage starch is deposited in amyloplasts of seeds, tubers, and roots for long-term storage.
2. **Biosynthesis:** involves ADP-glucose pyrophosphorylase (rate-limiting), starch synthases (elongate α -1,4 chains), branching enzymes (introduce α -1,6 bonds), and debranching enzymes (trim branches for granule crystallisation).
3. **Mobilisation:** catalysed by α -amylase (endoglycosidase), β -amylase (exoglycosidase), debranching enzyme (isoamylase/pullulanase), and α -glucan phosphorylase. During germination, gibberellin induces amylase synthesis in the aleurone layer.
4. **Human digestion:** salivary and pancreatic α -amylase hydrolyse internal α -1,4 bonds. Brush-border α -glucosidases (maltase-glucoamylase, sucrase-isomaltase) complete digestion to glucose. The α -linkages make starch a high-glycaemic-index carbohydrate.

1.4 Inulin

Structure

Inulin is a **fructan**, a polymer of D-fructofuranose (Fruf) units linked by **β -(2 $\rightarrow$ 1) glycosidic bonds**. The chain is usually terminated by a single α -(1 $\rightarrow$ 2)-linked D-glucopyranose residue (as in sucrose) at the reducing end.



The five-membered ring and β -2,1 linkage render it indigestible to humans.)

Biochemical Functions

- **Fructan storage reserve:** found in roots and tubers of Asteraceae (chicory, Jerusalem artichoke, dahlia). Synthesised from sucrose by **sucrose:sucrose 1-fructosyltransferase (1-SST)** and **fructan:fructan 1-fructosyltransferase (1-FFT)**.
- **Osmotic and cold-stress tolerance:** inulin is depolymerised to free fructose and sucrose under low temperatures, contributing to freezing-point depression and osmoregulation.
- **Dietary and prebiotic role:** human enzymes cannot cleave β -(2 $\rightarrow$ 1) bonds, so inulin reaches the colon intact, where it is selectively fermented by bifidobacteria and lactobacilli. The short-chain fatty acids (acetate, propionate, butyrate) produced support colonic health and mineral absorption.
- **Clinical diagnostic tool:** inulin is freely filtered by the glomeruli and is neither secreted nor reabsorbed by renal tubules. **Inulin clearance** (urine concentration $\times$ urine flow / plasma concentration) accurately measures the glomerular filtration rate (GFR), the gold standard for renal function assessment.

10 Theory-Based Questions and Answers: PLANT POLYSACCHARIDES

Plant Q1. Humans can digest starch but not cellulose, even though both are glucose polymers. Explain this difference at the molecular level.

Plant A1. Starch is composed of glucose units linked by α -glycosidic bonds (α -1,4 and α -1,6), which are recognised and hydrolysed by human α -amylases and brush-border α -glucosidases. Cellulose, in contrast, has β -1,4-glycosidic bonds. The alternating orientation of glucose residues produces a flat, ribbon-like structure that cannot fit into the catalytic grooves of mammalian digestive enzymes. Humans lack cellulases (β -1,4-glucanases), so cellulose remains undigested and passes through the gut as dietary fibre. Ruminants and termites digest cellulose through symbiotic bacteria and protists that produce cellulase enzyme complexes.

Plant Q2. Compare the structures of amylopectin and glycogen. How do the differences in branching relate to their biological roles?

Plant A2. Both are α -(1 $\rightarrow$ 4)-linked glucose polymers with α -(1 $\rightarrow$ 6) branches. Amylopectin has branches every 24–30 residues, while glycogen is more highly branched (every 8–12 residues). Amylopectin's clustered branching pattern, with alternating crystalline and amorphous lamellae, produces a semi-crystalline granule ideal for dense, long-term storage and relatively slow, controlled mobilisation during germination. Glycogen's extreme, uniform

branching yields a compact, highly soluble particle with thousands of non-reducing ends, enabling simultaneous attack by multiple glycogen phosphorylase molecules for extremely rapid glucose release suited to animal metabolic demands.

Plant Q3. Explain how the degree of methylesterification determines the gelation properties of pectin.

Plant A3. Pectin is synthesised in a highly methylesterified form (degree of esterification, DE > 50%). The methyl esters mask negative charges, preventing premature Ca^{2+} cross-linking. **High-methoxyl (HM) pectin** (DE > 50%) requires high sugar concentrations (>55%) and low pH (<3.5) to gel. The sugar dehydrates the pectin chains, and the acid suppresses charge repulsion, promoting hydrogen bonding and hydrophobic interactions between methyl ester groups. **Low-methoxyl (LM) pectin** (DE < 50%), produced by pectin methylesterase activity, has free carboxyl groups that cross-link via Ca^{2+} in the "egg-box" model, forming gels independent of sugar or acid. This allows LM pectin to be used in reduced-calorie and low-sugar products.

Plant Q4. Describe the biochemical pathway of starch mobilisation in a germinating seed, including the hormonal trigger.

Plant A4. During germination, the embryo synthesises and releases **gibberellin (GA)**, which diffuses to the aleurone layer surrounding the endosperm. GA acts as a signalling molecule, inducing gene expression for hydrolytic enzymes, primarily **α -amylase**. α -Amylase is secreted into the endosperm and acts as an endoglycosidase, cleaving internal α -1,4 bonds in starch granules to release soluble dextrans and oligosaccharides. **β -Amylase** (exoglycosidase) cleaves maltose from non-reducing ends, while **debranching enzyme** (isoamylase) cleaves α -1,6 branch points. **α -Glucan phosphorylase** releases glucose-1-phosphate. The resulting maltose and glucose are transported to the growing embryo as carbon and energy sources.

Plant Q5. Inulin clearance is the gold standard for measuring glomerular filtration rate (GFR). What biochemical properties of inulin make it ideal for this purpose?

Plant A5. Inulin possesses a unique combination of properties: (a) it is **freely filtered** across the glomerular capillaries due to its moderate molecular weight (~5 kDa) and compact helical structure; (b) it is **not reabsorbed** by renal tubular cells; (c) it is **not secreted** into the tubular lumen; (d) it is **not metabolised**, synthesised, or produced endogenously by the kidney; and (e) it is **physiologically inert** and non-toxic. Because of these properties, the amount of inulin excreted in the urine exactly equals the amount filtered through the glomeruli. Therefore, inulin clearance ($U_{\text{inulin}} \times V / P_{\text{inulin}}$) provides a direct and accurate measurement of the GFR.

Plant Q6. What is the structural basis of cellulose's tensile strength and why is it insoluble in water despite being composed of glucose?

Plant A6. Cellulose's tensile strength arises from its β -(1→4)-linked **glucan chains**, which adopt a flat, ribbon-like conformation. This conformation permits extensive **intra-chain hydrogen bonds** (O-3'-O-5 and O-6-O-2') that rigidify each chain and **inter-chain hydrogen bonds** (O-6-O-3') that tightly pack dozens of parallel chains into highly ordered, crystalline **microfibrils**. The hydroxyl groups responsible for water solubility in free glucose are engaged in this dense hydrogen-bonding network within and between the chains. This crystalline supramolecular structure excludes water molecules from the core of the microfibril, making cellulose insoluble and chemically resilient, perfect for its structural role.

Plant Q7. How does the clustering of branches in amylopectin contribute to the formation of starch granule crystallinity?

Plant A7. The branch points in amylopectin are not randomly distributed but are clustered in specific regions. The linear α -1,4 chains between branch points within clusters can align and form parallel double helices that pack into ordered, **crystalline lamellae** (5–6 nm thick). The regions dense with α -1,6 branch points form **amorphous lamellae** (2–3 nm thick) that are less ordered. Alternating crystalline and amorphous lamellae (with a 9 nm repeat) create the blocklet structure and the semicrystalline nature of starch granules, visible as the Maltese cross under polarised light. Debranching enzymes are essential during synthesis to remove inappropriately placed branches that would otherwise disrupt this crystallisation.

Plant Q8. What is the role of expansins in cell wall loosening, and how does this relate to cellulose structure?

Plant A8. Expansins are cell wall proteins that mediate **acid-induced cell wall loosening** without hydrolytic activity. They are hypothesised to disrupt the **hydrogen bonds** between cellulose microfibrils and matrix polysaccharides, particularly xyloglucan. By breaking these non-covalent bonds locally, expansins allow cellulose microfibrils and the associated matrix to slip past one another under turgor pressure, enabling cell expansion. This mechanism is pH-dependent; it is activated by auxin-induced proton extrusion into the cell wall. Because expansins do not cleave covalent bonds, they loosen the wall reversibly and without compromising long-term structural integrity, a neat adaptation leveraging cellulose's non-covalent supramolecular assembly.

Plant Q9. How do oligogalacturonides (OGs) function as signalling molecules in plant defence?

Plant A9. Oligogalacturonides (OGs) are fragments of homogalacturonan, typically with a degree of polymerisation of 10–15, released from the cell wall by the action of pathogen-derived polygalacturonases during infection. These OGs act as **damage-associated molecular patterns (DAMPs)**. They are recognised by **wall-associated kinases (WAKs)**, a family of receptor-like kinases at the plasma membrane. Binding of OGs to WAKs triggers a signal transduction cascade involving mitogen-activated protein kinases (MAPKs), leading to a rapid burst of reactive oxygen species (ROS), activation of defence gene expression, production of antimicrobial phytoalexins, and cell wall reinforcement. This system allows plants to detect and respond to pathogenic wall degradation early in infection.

Plant Q10. Contrast the synthesis and energy storage mechanisms of starch and inulin in plants.

Plant A10. Starch is a glucan synthesised in plastids (chloroplasts for transient starch, amyloplasts for storage starch) using **ADP-glucose** as the activated sugar donor. The polymer is deposited as semi-crystalline granules and, upon mobilisation, yields glucose that is directly metabolised via glycolysis. **Inulin** is a fructan synthesised in the vacuole from **sucrose** by the action of fructosyltransferases (1-SST and 1-FFT). It is a soluble, non-crystalline polymer. Mobilisation involves fructan exohydrolases (FEHs) that release fructose, which must be phosphorylated before entering central metabolism. Inulin's synthesis in the vacuole compartmentalises it away from cellular metabolism, linking its accumulation to vacuolar osmotic functions, whereas starch synthesis in the plastid is directly tied to the photosynthetic carbon reduction cycle.

2. ANIMAL CARBOHYDRATE MACROMOLECULES

2.0 Introduction: Overview of Animal Polysaccharides

Animals utilise polysaccharides for energy homeostasis, structural support in specialised tissues, and extracellular matrix (ECM) organisation. Glycosaminoglycans (GAGs) are particularly prominent in signalling and mechanical functions. Listed below are the major animal polysaccharides in approximate order of biological indispensability.

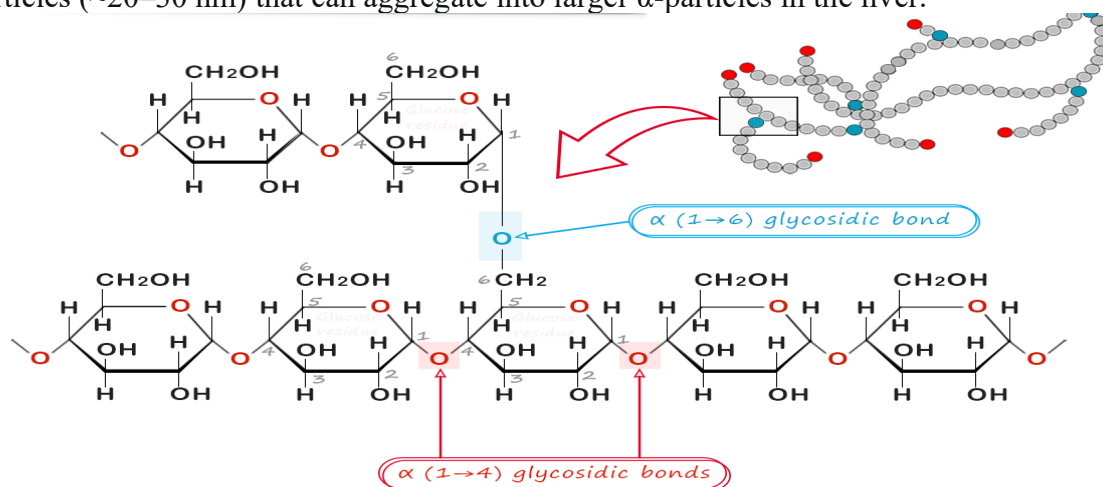
1. **Glycogen** – The prime short-term, rapidly mobilisable energy buffer for all animal cells; central to blood glucose homeostasis.
2. **Glycosaminoglycans (GAGs) & Proteoglycans** – A diverse family that forms the hydrated gel of the ECM, providing tissue turgor, shock absorption, and signalling (includes hyaluronic acid, chondroitin sulphate, heparan sulphate).
3. **Chitin** – The defining structural polymer of the arthropod exoskeleton; second most abundant biopolymer globally.

4. **Heparin** – A highly sulphated GAG stored in mast cell granules with potent anticoagulant function.
5. **Heparan Sulphate** – A GAG related to heparin, present on cell surfaces and in the ECM, with crucial roles in cell signalling, adhesion, and development.
6. **Chondroitin Sulphate** – A sulphated GAG in cartilage (as aggrecan), providing compressive resistance in joints.
7. **Keratan Sulphate** – A sulphated GAG in cornea, cartilage, and bone, maintaining tissue hydration.
8. **Dermatan Sulphate** – A GAG essential for the structural integrity of skin, blood vessels, and heart valves.

2.1 Glycogen

Structure

Glycogen is a highly branched homopolysaccharide of glucose. It is built on a core protein, **glycogenin**, and consists of linear chains of **α -(1 $\rightarrow$ 4)-linked D-glucose** with frequent **α -(1 $\rightarrow$ 6) branch points** every 8–12 residues. This dense branching creates spherical β -particles (~20–30 nm) that can aggregate into larger α -particles in the liver.



(The extreme branching produces thousands of non-reducing ends per molecule, accessible to glycogen phosphorylase.)

Biochemical Functions

1. Energy Storage and Release: The dense branching creates a huge number of non-reducing ends. Glycogen phosphorylase simultaneously cleaves many α -1,4 bonds, releasing glucose-1-phosphate in a burst to meet high energy demands. Glycogen functions primarily as a rapid-mobilization energy reserve and blood sugar buffer, with its main stores located in the liver and skeletal muscles

- **Liver glycogen reserve:** Acts as a whole-body glucose reservoir, breaking down to release glucose into the bloodstream during fasting or between meals to maintain stable blood sugar levels. It stores up to ~100 g. Glucose-6-phosphatase enables the

production of free glucose for export into the blood, maintaining blood glucose homeostasis between meals. Liver phosphorylase is allosterically inhibited by glucose and hormonally activated by glucagon and adrenaline via cAMP-dependent phosphorylation.

- **Muscle glycogen reserve:** Supplies localized metabolic fuel exclusively for skeletal muscle cells during active contraction and exercise. It stores up to ~400 g. Muscle lacks glucose-6-phosphatase, so glycogen-derived Glucose-1-Phosphate enters glycolysis directly for local ATP production. Muscle phosphorylase is allosterically activated by AMP (low energy status) and Ca^{2+} -calmodulin (contraction signal), coupling glycogenolysis to contractile activity.
- **Compact structure:** glycogen features a highly branched polymer design that allows enzymes to quickly attach or detach glucose molecules as energy demands shift. Glycogen synthase (active when dephosphorylated by insulin signalling) and glycogen phosphorylase (active when phosphorylated by glucagon/adrenaline cascade) are reciprocally regulated. Glycogen storage diseases (e.g., von Gierke, McArdle) from enzyme deficiencies underscore metabolic reliance on glycogen.

2. Cellular and Specialized Functions

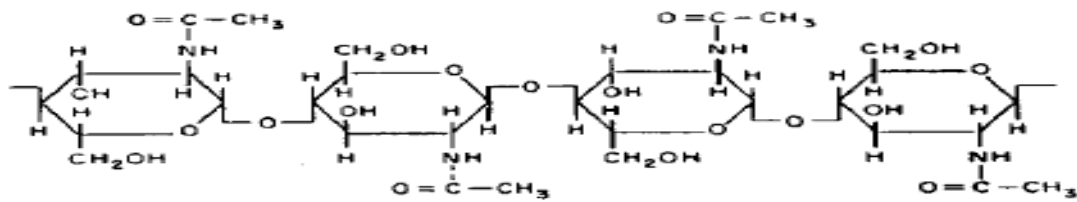
- **Osmotic balance:** Remains insoluble in cellular water, preventing the unwanted osmotic pressure changes that storing equivalent free glucose molecules would cause.
- **Brain and nervous support:** Maintains small glycogen reserves in astrocytes to protect against sudden drops in blood sugar and assist local brain metabolism.
- **Fetal lung development:** Accumulates in specialized lung cells late in pregnancy to help synthesize pulmonary surfactant required for breathing after birth.

2.2 Chitin

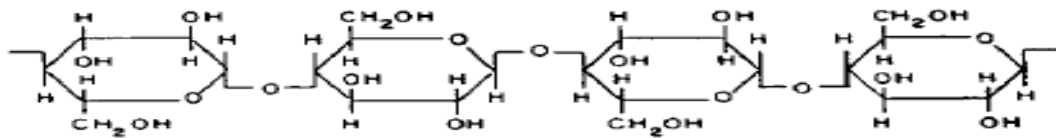
Structure

Chitin is a linear homopolymer of **N-acetyl-D-glucosamine (GlcNAc)** units joined by **β -(1→4) glycosidic bonds**, structurally analogous to cellulose. Chitin is a tough, natural sugar polymer that forms the main structural support in insect shells and fungal walls. Adjacent chains run antiparallel in the dominant α -chitin form, assembling into microfibrils that associate with proteins and sometimes calcium carbonate.

CHITIN



CELLULOSE



(GlcNAc = N-acetylglucosamine. The acetamido group strengthens inter-chain H-bonds, making chitin even more chemically resistant than cellulose.)

Biochemical Functions

1. Structural Support and Protection

- **Arthropod Exoskeletons:** Forms hard outer shells for insects, spiders, and crustaceans like crabs and shrimp, protecting them from injury and stopping water loss.
- **Fungal Cell Walls:** Replaces cellulose in fungi to give cells strength, stability, and fixed shape.
- **Invertebrate Anatomy:** Builds tough parts like mollusk radulae and squid beaks.

2. Ecological and Nutritional Roles

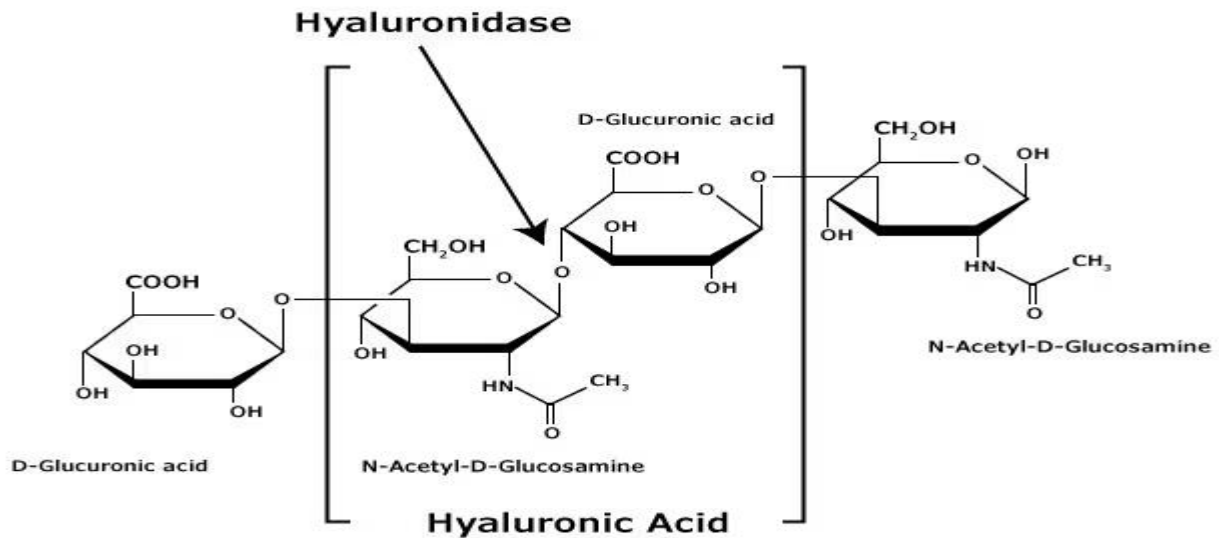
- **Nutrient Cycling:** Acts as a major renewable source of carbon and nitrogen in oceans and soil when broken down by specialized bacteria.
- **Dietary Fiber:** Serves as an indigestible fiber for animals that consume it, adding bulk and supporting gut health.
- **Biomedical applications:** chitin and its deacetylated form **chitosan** are biocompatible, biodegradable, and wound-friendly. Chitosan, with its positive charge, promotes haemostasis (platelet aggregation), has antimicrobial properties, and serves as a scaffold for tissue engineering and mucoadhesive drug delivery.

2.3 Hyaluronic Acid (Hyaluronan, HA)

Structure

Hyaluronic acid is a linear, non-sulphated glycosaminoglycan (GAG). It is a heteropolysaccharide with a repeating disaccharide unit: **β-D-glucuronic acid (GlcA) linked**

(1→3) to β -D-N-acetylglucosamine (GlcNAc). These units are then connected by a β -(1→4) linkage to the next disaccharide. It reaches molecular masses $>10^6$ Da.



(Glucuronic acid. The alternating β -1,3 and β -1,4 linkages give the polymer a unique, flexible random coil structure.)

Biochemical Functions

1. Hydration and Structure

- **Water retention:** Hyaluronic acid, with its massive hydrodynamic volume and copious bound water, provides tissue turgor. It keeps tissues moist by binding large amounts of water. It is very good at retaining water. A quarter-teaspoon of hyaluronic acid holds about one and a half gallons of water.
- **Skin elasticity:** Maintains skin firmness, structure, and a smooth appearance in the extracellular matrix.
- **Ocular support:** Preserves the shape and fluid viscosity inside the eye's vitreous humor.

2. Movement and Protection

- **Joint lubrication:** Acts as a shock absorber and lubricant in joint synovial fluid to prevent bones from grinding.
- **Cell signaling:** Influences cell movement, growth, and how tissues respond during development.

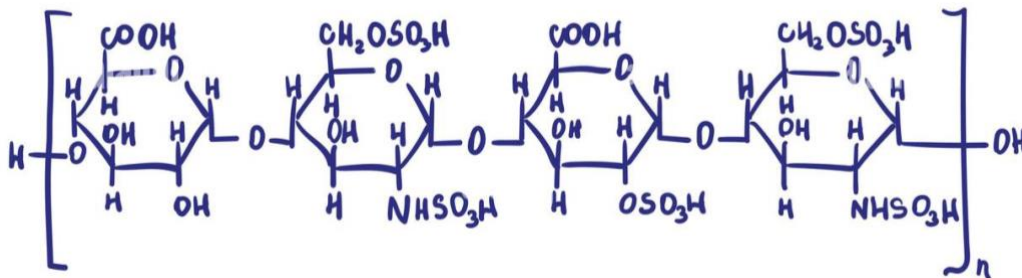
3. Healing and Repair

- **Wound management:** Controls inflammation levels and helps new blood vessels form to repair damaged areas.
- **Immune regulation:** Changes its behavior based on molecule size, where large chains block inflammation and smaller fragments stimulate immune repair responses.

2.4 Heparin

Structure

Heparin is a highly sulphated, linear glycosaminoglycan. Its major repeating disaccharide is: **α -L-iduronic acid-2-sulphate (IdoA2S)** linked (1 $\rightarrow$ 4) to **α -D-glucosamine-N,6-disulphate (GlcNS6S)**. The polymer is covalently attached to a **serine residue** of the core protein serglycin in mast cell granules and released as free chains (5–40 kDa).



Biochemical Functions

1. Anticoagulation and Clot Regulation

- Due to its unique pentasaccharide sequence, heparin exerts anticoagulant activity after binding with antithrombin, inhibits the activation of factors Xa and IIa in the coagulation cascade.
- It accelerates the power of antithrombin III to block thrombin (Factor IIa) and Factor Xa, which keeps blood smooth and stops dangerous new clots from growing.
- Unlike other drugs, natural heparin does not break down existing clots; it only lets the body's natural systems manage them safely.

2. Storage and Cellular Defense

- Stored in the granules of mast cells located near blood vessel walls in tissues like the liver and lungs.
- Released during tissue injury to serve as a local defense mechanism against foreign invaders and bacteria.
- Possesses the highest negative charge density of any known biological molecule, helping it attach to and control various active proteins during inflammation.

10 Theory-Based Questions and Answers: ANIMAL POLYSACCHARIDES

Animal Q1. Why does the highly branched structure of glycogen allow for much faster glucose release than would a linear polymer of the same size?

Animal A1. Glycogenolysis is catalysed by glycogen phosphorylase, which sequentially cleaves α -1,4 bonds only from non-reducing ends, releasing glucose-1-phosphate. A linear polymer with no branches would have only a single non-reducing end, creating a strict bottleneck for glucose release. Glycogen's abundant α -1,6 branch points create one new non-reducing end per branch, resulting in thousands of ends on a single molecule. This allows many phosphorylase molecules to act simultaneously. This "many-fronts" enzymatic attack enables a burst of glucose-1-phosphate production, rapidly fuelling high-energy demands such as intense muscle contraction or the hepatic response to acute hypoglycaemia.

Animal Q2. Describe the precise molecular mechanism by which heparin accelerates antithrombin III activity. Why can low-molecular-weight heparins (LMWHs) only inhibit factor Xa?

Animal A2. Heparin accelerates antithrombin III (AT-III) activity through a specific high-affinity pentasaccharide sequence that includes a critical **3-O-sulphated glucosamine** residue. Binding of this pentasaccharide induces a conformational change in AT-III, repositioning its reactive centre loop to become a highly efficient inhibitor of **factor Xa**. For **thrombin (factor IIa)** inhibition, an additional non-specific electrostatic binding is required. Heparin must form a ternary bridge: the pentasaccharide sequence binds AT-III, and a contiguous, negatively charged stretch of ≥ 13 additional saccharide units binds thrombin, forming an AT-III-heparin-thrombin ternary complex. LMWHs are predominantly shorter than 18 saccharides and thus lack the minimum chain length needed for this bridging. They retain the pentasaccharide sequence and therefore catalyse factor Xa inhibition but cannot catalyse thrombin inhibition.

Animal Q3. Chitin serves as a structural polymer both in arthropod exoskeletons and fungal cell walls. How does its biological context and organisation differ in these two organisms?

Animal A3. In **arthropods**, chitin is synthesised by epidermal cells and deposited in the extracellular space as part of a complex stratified cuticle. It associates with cuticular proteins, is often hardened by sclerotisation (cross-linking with phenols/quinones), and in crustaceans, is heavily mineralised with calcium carbonate. The chitin-protein microfibrils form a helicoidal layering (Bouligand structure) providing exceptional toughness. This exoskeleton is periodically moulted under hormonal control. In **fungi**, chitin is a key component of the cell wall, deposited primarily at the growing hyphal tip and the septum by chitin synthase. It associates with β -glucans and glycoproteins, forming a dynamic, flexible wall that resists turgor pressure. Fungal chitin is not calcified, and its recognition by host receptors as a PAMP is a crucial element of innate immunity against fungal pathogens.

Animal Q4. Hyaluronic acid in synovial fluid acts as a lubricant and shock absorber. Explain how its high molecular mass and physical properties make this dual function possible.

Animal A4. Hyaluronic acid (HA) in synovial fluid exists at $>10^6$ Da, forming an extended, heavily hydrated random coil that occupies a hydrodynamic volume ~ 1000 times its dry polymer volume. At **low shear rates** (e.g., during standing or slow walking), the long HA chains entangle extensively, forming a continuous viscoelastic network. This network behaves as an elastic solid, dissipating compressive forces and providing a gel-like cushion between cartilage surfaces. At **high shear rates** (e.g., during a sprint), the entangled chains align in the direction of flow and disentangle. This produces a dramatic, reversible drop in viscosity (shear-thinning/pseudoplasticity), allowing the fluid to serve as a low-friction lubricant, minimising wear and energy loss during rapid joint movement. The HA-aggreCAN aggregates further enhance this via fixed swelling pressure.

Animal Q5. Liver glycogen and muscle glycogen serve different physiological roles. Explain how the biochemical difference in their enzyme complement (specifically glucose-6-phosphatase) underlies this functional divergence.

Animal A5. The key enzymatic difference between liver and muscle is the presence of **glucose-6-phosphatase**. **Liver** contains glucose-6-phosphatase in the endoplasmic reticulum, which cleaves the phosphate from glucose-6-phosphate (G-6-P) produced during glycogenolysis. This enables the liver to release **free glucose** into the bloodstream, serving as the primary homeostatic organ that maintains blood glucose levels for other tissues, especially the brain. **Skeletal muscle** lacks glucose-6-phosphatase. Consequently, G-6-P derived from muscle glycogen cannot be dephosphorylated and exported. Instead, it is directly shunted into the glycolytic pathway to generate ATP locally for muscle contraction. Muscle glycogen is thus a selfish, private fuel reserve, whereas liver glycogen is a communal, altruistic store for the entire body.

Animal Q6. What structural feature of chitin makes it indigestible by human digestive enzymes, and how does this compare to cellulose?

Animal Q6. Chitin is composed of N-acetyl-D-glucosamine (GlcNAc) units linked by **β -(1 $\rightarrow$ 4) glycosidic bonds**, structurally identical in backbone to cellulose (which is β -1,4-glucose). Human digestive enzymes, such as salivary and pancreatic α -amylases, are specific for **α -glycosidic linkages** (as in starch) and cannot hydrolyse β -linkages. Mammals generally do not produce chitinases (β -1,4-poly-GlcNAc hydrolases) or cellulases in significant quantities. Therefore, both chitin and cellulose are resistant to the human digestive

tract. However, some mammals (e.g., insectivores, certain primates) have been found to express acidic chitinases (like AMCase) in the stomach, suggesting a limited ability to digest chitin from insect exoskeletons, whereas cellulase production is almost exclusively microbial.

Animal Q7. Compare the structure and charge density of hyaluronic acid and heparin. How do these differences relate to their respective biological functions?

Animal A7. Hyaluronic acid (HA) is a non-sulphated GAG with a repeating disaccharide of GlcA and GlcNAc. It has one carboxyl group per disaccharide, giving a moderate, uniform negative charge density. This charge attracts a large hydration shell, making HA an ideal space-filling, hydrating, and lubricating molecule for ECM and synovial fluid. Its binding interactions are broad and largely nonspecific, often involving multivalent receptor clustering (e.g., CD44). **Heparin** is the most highly negatively charged biopolymer in nature, with up to 3–4 sulphate groups per disaccharide on a backbone of IdoA(2S) and GlcNS(6S). This extreme charge density provides high-affinity, sequence-specific electrostatic interactions with proteins like antithrombin III and growth factors. Heparin's structure has evolved for precise, potent, and tightly regulated pharmacological-like protein binding, a contrast to HA's more physical, hydrating role.

Animal Q8. What is the biological rationale for the extremely large size of hyaluronic acid (up to millions of Daltons) compared to other GAGs?

Animal A8. The extreme molecular weight of HA is directly linked to its primary extracellular functions as a space-filling, hydrating, and organising molecule. A single high-molecular-weight HA chain, via its enormous hydrated random coil, can occupy and organise a vast volume of extracellular space, controlling tissue hydration and porosity. It can simultaneously interact with many copies of cell surface receptors (e.g., CD44), forming a pericellular coat, and organise proteoglycan aggregates at a supramolecular scale. Synthesised at the plasma membrane by HA synthases (HAS) and extruded directly into the extracellular space, its large size is not constrained by intracellular packaging. Lower molecular weight GAGs like heparan sulphate or chondroitin sulphate are synthesised on core proteins intracellularly and function on a more local, nanoscale level, presenting specific sulphation motifs for fine-tuned protein binding.

Animal Q9. Explain the role of glycogenin in glycogen synthesis. What would happen if a cell tried to synthesise glycogen without it?

Animal A9. Glycogenin is the core primer protein for glycogen synthesis. It is an autocatalytic enzyme that catalyses the transfer of a glucose residue from UDP-glucose to a specific tyrosine residue (Tyr-194) on itself. It then extends this chain to about 8–10

α -1,4-linked glucose residues, forming a short oligosaccharide primer still attached to the glycogenin protein. This glycogenin-oligosaccharide complex is the essential substrate for **glycogen synthase**, which cannot initiate a new glycogen chain de novo. Glycogen synthase can only elongate existing α -1,4 chains. Without glycogenin to provide the initial primer, glycogen synthase would have no substrate, and a cell would be entirely incapable of initiating glycogen synthesis. The cell would thus lose its primary short-term energy reservoir.

Animal Q10. How does chitosan differ structurally from chitin, and why does this structural difference make chitosan more versatile for biomedical applications?

Animal Q10. Chitin is a homopolymer of N-acetyl-D-glucosamine (GlcNAc) with acetamido groups at C-2. **Chitosan** is the N-deacetylated derivative, where the degree of deacetylation is typically >50%, converting most GlcNAc residues to **D-glucosamine (GlcN)** with free amino ($-\text{NH}_2$) groups at C-2. This structural change has profound physicochemical consequences. The free amino groups have a pKa around 6.3–6.5, meaning chitosan becomes protonated and **polycationic** under slightly acidic physiological conditions. This positive charge enables chitosan to bind to negatively charged cell surfaces and mucin (mucoadhesive), disrupt bacterial membranes (antimicrobial), complex with DNA for gene delivery, and electrostatically interact with negatively charged drug molecules. Chitin, being neutral and highly crystalline, lacks these soluble, interactive properties, making chitosan far more versatile for biomedical applications like wound dressings, drug delivery, and tissue engineering scaffolds.

3. MICROBIAL CARBOHYDRATE MACROMOLECULES

3.0 Introduction: Overview of Microbial Polysaccharides

Microorganisms produce a vast array of structurally unique polysaccharides, predominantly as extracellular capsules (exopolysaccharides, EPS) or cell envelope components. Their biological roles are critical for structural integrity, pathogenicity, and biofilm formation. Listed below are the major microbial polysaccharides in approximate order of biological significance.

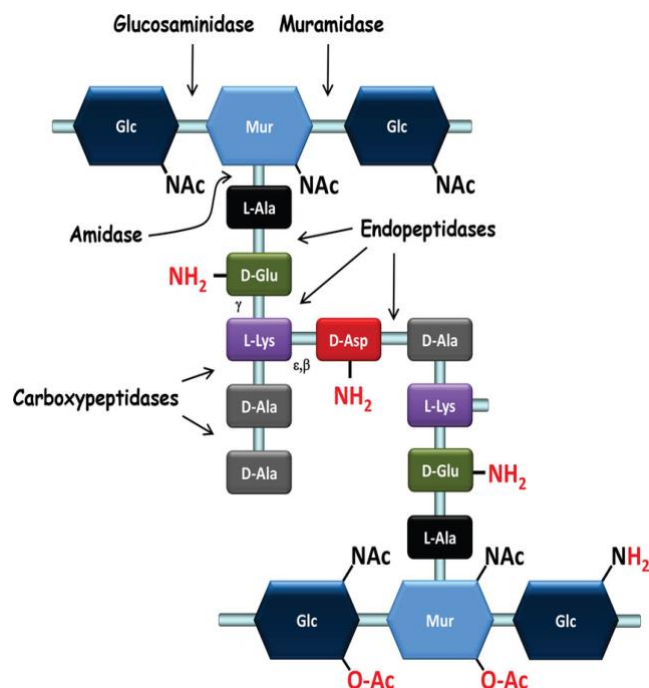
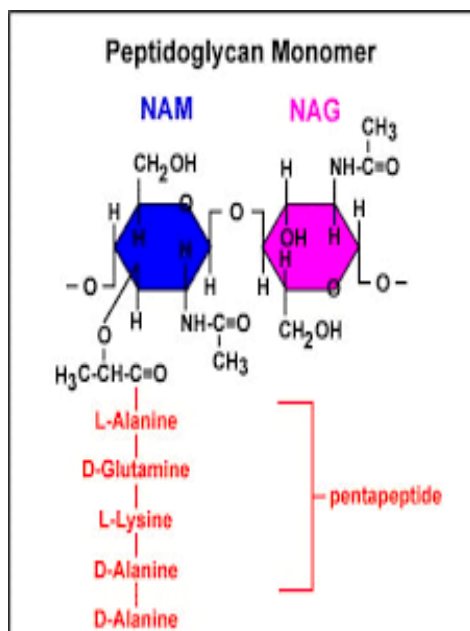
1. **Peptidoglycan (Murein)** – The definitive structural macromolecule of the bacterial cell wall; essential for osmotic survival and the single most important target of clinical antibiotics.
2. **Lipopolysaccharide (LPS)** – The defining Gram-negative outer membrane component and a potent endotoxin (lipid A) responsible for septic shock.
3. **Teichoic & Lipoteichoic Acids** – Phosphate-rich polymers essential for cell division, metal ion homeostasis, and autolytic regulation in Gram-positive bacteria.

4. **Alginate** – An acetylated mannuronic/guluronic acid polymer forming the biofilm matrix of *Pseudomonas aeruginosa* in chronic infections (e.g., cystic fibrosis).
5. **Dextran** – An α -glucan produced by oral streptococci that mediates dental plaque biofilm formation and has widespread industrial chromatography use.
6. **Xanthan Gum** – A complex exopolysaccharide from *Xanthomonas* spp. with extraordinary shear-thinning rheology, integral to plant-pathogenic biofilm formation.
7. **Gellan Gum** – A chemically defined, cation-responsive gelling agent from *Spingomonas elodea* that forms thermoreversible gels.
8. **Curdlan** – A linear β -1,3-glucan that forms irreversible, firm gels upon heating, significant in bacterial adhesion and as a functional food ingredient.

3.1 Peptidoglycan (Murein)

Structure

Peptidoglycan is a heteropolymer forming a single, continuous mesh-like sacculus. The **glycan backbone** consists of alternating β -(1 $\rightarrow$ 4)-linked **N-acetylglucosamine (GlcNAc)** and **N-acetylmuramic acid (MurNAc)**. A **stem peptide** (e.g., L-Ala-D-Glu-m-DAP-D-Ala-D-Ala) is attached to the lactyl group of MurNAc. Adjacent stem peptides are cross-linked, either directly (Gram-negative) or via a pentaglycine bridge (Gram-positive).



Biochemical Functions of peptidoglycan

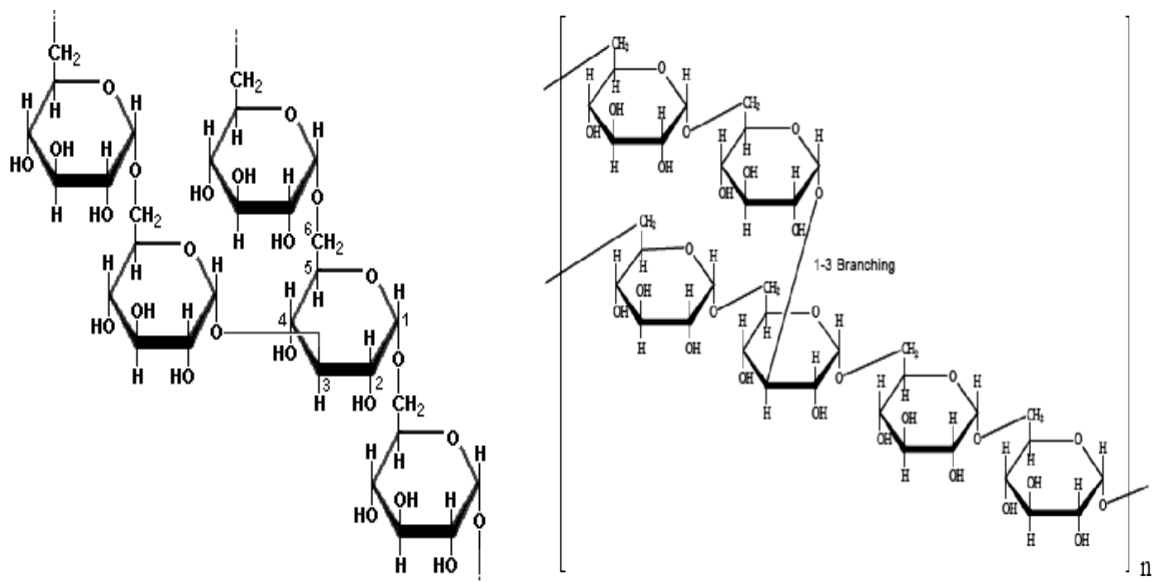
1. **Osmotic Protection:** Counteracts high internal cytoplasmic pressure, preventing the bacterial cell from bursting (*lysis*) in watery environments.
2. **Structural Rigidity:** Acts as a strong mechanical backbone that gives the cell wall its overall strength and resilience against external physical stress.

3. **Cell Division:** Plays a critical role in forming the septum (dividing wall) during binary fission so bacteria can successfully reproduce.
4. **Immune System Trigger:** Acts as a pathogen-associated molecular pattern (PAMP) that is recognized by host immune receptors (like Toll-like receptor 2), stimulating immune responses during an infection.

3.2 Dextran

Structure

Dextran is a branched homopolysaccharide produced mainly by lactic acid bacteria (*Leuconostoc mesenteroides*, *Streptococcus mutans*). Its primary backbone consists of α -(1→6)-linked D-glucose residues. Branches are introduced via α -(1→3), α -(1→2), or α -(1→4) linkages, depending on the bacterial strain and its glucosyltransferases (Gtfs). Molecular weight varies from 10^4 to 10^7 Da.



(The long α -1,6 backbone is flexible and soluble; the short branches create an adhesive, water-insoluble matrix.)

Biochemical Functions of Dextrans

Physiological and Medical Functions

1. **Plasma Volume Expansion:** High-molecular-weight dextrans hold large amounts of water in the blood, raising blood volume to treat shock or severe blood loss.
2. **Microvascular Flow Improvement:** Dextran 40 decreases blood viscosity and red blood cell clumping, which helps blood move smoothly through tiny vessels.
3. **Anti-Clotting (Antithrombotic) Action:** They bind to red blood cells and platelets to reduce stickiness and interfere with clotting proteins like Factor VIII and von Willebrand factor.
4. **Anti-Inflammatory Effects:** They help protect cells lining blood vessels from damage caused by active white blood cells during trauma or reperfusion.

Cellular and Research Functions

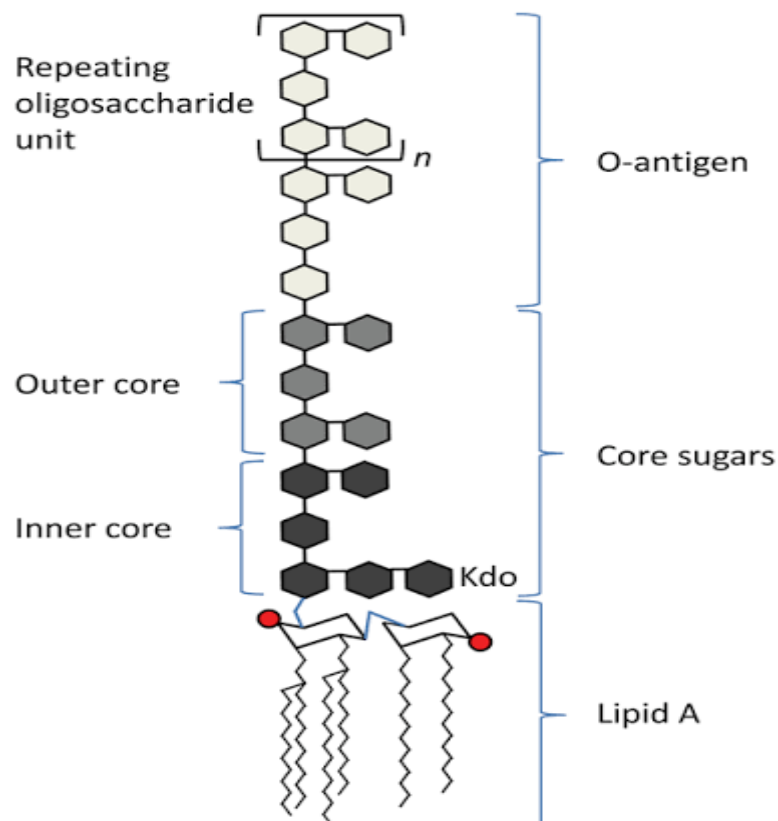
5. **Osmotic Stress:** Researchers use dextrans to apply controlled osmotic pressure to isolated cells and biological molecules.
6. **Endosomal Tracking:** Fluorescently labeled dextrans help scientists visualize and study early endosomes under a microscope.
7. **Vascular Permeability Testing:** Engineered vessel networks are perfused with dextran solutions to measure how substances leak across blood vessel walls.

Industrial and clinical tools: cross-linked dextran beads (**Sephadex**) are the classic media for size-exclusion (gel-filtration) chromatography. Dextran solutions (e.g., dextran 40) are used as plasma volume expanders in hypovolaemic shock due to their colloidal osmotic effect. Dextran sulphate is used experimentally as an anticoagulant and antiviral agent.

3.3 Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS), also known as endotoxin, is a large molecule found in the outer membrane of Gram-negative bacteria like *E. coli* and *Salmonella*. LPS consists of three main parts:

- a) **Lipid A:** A hydrophobic fat region that anchors the molecule and triggers strong toxicity and immune reactions.
- b) **Core Polysaccharide:** A middle sugar chain that connects Lipid A to the outer part.
- c) **O-Antigen:** A repeating sugar chain on the outside that helps bacteria identify and evade host defenses.



Biological functions of lipopolysaccharide

- a) **Bacterial Protection:** It gives the outer membrane stability and protects bacteria from harmful chemicals like bile salts.
- b) **Immune Response:** It acts as a major warning sign for the body's immune system, alerting white blood cells to infection. Lipopolysaccharide is a potent stimulator of the vertebrate innate immune system. This innate immune system, mediated by macrophages and dendritic cells, generates a rapid response to infectious agents. Structural patterns common to diverse LPS molecules are recognized by Toll-like receptors (TLR) and accessory proteins in serum. LPS released from bacterial membranes is bound to LPS binding protein (LBP) in serum, transferred to CD 14, an LPS receptor glycoprotein, and presented to the TLR-4-MD-2 complex, stimulating production of cytokines.
- c) **Inflammation and Shock:** Large amounts released into the bloodstream can cause dangerous inflammation, high fever, low blood pressure, and septic shock.

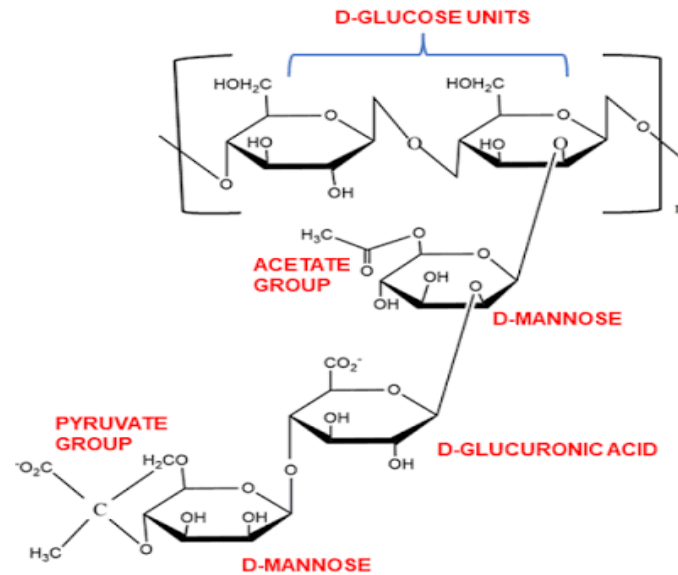
Research and drug development:

LPS has a wide range of uses in research and drug development. It may be used to stimulate immune cells and investigate the innate immune response. In drug development, structurally modified LPS forms, such as Lipid A, have been used as vaccine adjuvants. LPS-derived oligosaccharides have been conjugated to carrier proteins in the development of LPS containing human vaccines.

3.4 Xanthan Gum

Xanthan gum is a complex polysaccharide, extracellularly branched and produced by *Xanthomonas campestris*. Its structure comprises of many smaller sugar molecules bonded together.

- a) **Main Chain:** features a cellulosic backbone made of β -(1 $\rightarrow$ 4)-linked D-glucose units **backbone** (the same basic building block found in plant cellulose).
- b) **Side Chains:** Attached to the main chain is a repeating trisaccharide (three-sugar) side chain composed of mannose and glucuronic acid (**D-Manp-(1 $\rightarrow$ 4)- β -D-GlcpA-(1 $\rightarrow$ 2)- α -D-Manp.**).
- c) **Decorations:** These side chains are further decorated with non-carbohydrate groups like acetyl and pyruvate residues. The terminal mannose often carries a **pyruvate** (4,6-O-ketal) group, and the inner mannose is frequently **O-acetylated** at C-6.



(The stiff, negatively charged side chains are crucial for viscosity.)

Physiological and Biological Interactions

1. **Plant pathogen biofilm:** *Xanthomonas* species are phytopathogens causing black rot and wilt diseases. Xanthan gum encapsulates the bacteria, protecting them from desiccation and host antimicrobial compounds. It also physically blocks xylem vessels, contributing to wilting symptoms and aiding in colonisation.
2. **Extraordinary rheology:** in aqueous solution, xanthan molecules adopt a rigid, ordered double-helical conformation. The stiff chains and their high molecular weight form an entangled network with extremely high **low-shear viscosity**. Under high shear (pouring, pumping), the chains align and disentangle, causing a reversible drop in viscosity called **pseudoplasticity (shear-thinning)**. This property is stable across wide ranges of pH, temperature, and salt concentration.
3. **Industrial thickener and stabiliser:** harnessed in salad dressings, sauces, ice cream (prevents ice crystal formation), gluten-free baked goods, cosmetics, and oil-drilling fluids to provide suspension, pourability, and cling.
4. **Synergistic gelation:** xanthan forms strong, thermally reversible gels when mixed with galactomannans (e.g., locust bean gum) or glucomannans (e.g., konjac). The unsubstituted mannan backbone regions of the partner polysaccharide interact with the xanthan side chains, creating mixed junction zones that form a cohesive gel network.
5. **Soluble Fiber Properties:** Because it is a high-molecular-weight polysaccharide, human enzymes cannot break it down. It absorbs water in the digestive tract to form a viscous, gel-like matrix.
6. **Blood Glucose Modulation:** The high viscosity slows gastric emptying and the intestinal absorption of glucose, which helps blunt postprandial (post-meal) blood sugar spikes.

7. **Prebiotic and Microbiome Effects:** Specific gut bacteria (such as strains from the family *Ruminococcaceae*) can ferment xanthan gum. This microbial breakdown yields **short-chain fatty acids (SCFAs)** like butyrate, which support intestinal barrier homeostasis and cellular health.
8. **Satiety and Lipid Metabolism:** By delaying digestion and expanding in the gut, it can increase subjective feelings of fullness and potentially assist in cholesterol regulation at higher intakes. [[1](#)]

10 Theory-Based Questions and Answers: MICROBIAL POLYSACCHARIDES

Microbial Q1. Lysozyme and penicillin both target bacterial peptidoglycan but by entirely different mechanisms. Contrast these mechanisms.

Microbial A1. Lysozyme is a glycoside hydrolase (specifically, a muramidase). It hydrolyses the β -(1 $\rightarrow$ 4) glycosidic bond between MurNAc and GlcNAc in the pre-existing glycan backbone of the peptidoglycan sacculus. This depolymerisation of the protective mesh leads to weakening and osmotic lysis of the cell. **Penicillin** (and other β -lactams) does not act on existing peptidoglycan. It is a structural analogue of the D-Ala-D-Ala terminus of the stem peptide. It irreversibly acylates the active-site serine of **transpeptidase penicillin-binding proteins (PBPs)**, blocking the cross-linking step of new peptidoglycan synthesis. This means bacteria continue to synthesise un-cross-linked, weakened cell wall and eventually lyse as they grow, but penicillin does not degrade the existing sacculus.

Microbial Q2. How do oral streptococci use dextran to cause dental caries? Detail the enzyme involved and its substrate.

Microbial A2. Oral streptococci, particularly *Streptococcus mutans*, secrete extracellular **glucosyltransferases (Gtfs)**. These enzymes bind to the acquired pellicle on the tooth surface. Gtfs catalyse a transglycosylation reaction using **sucrose** (table sugar) as their specific substrate. They cleave the high-energy α -1, β -2 glycosidic bond of sucrose and polymerise the glucose moiety into **dextran**, an α -(1 $\rightarrow$ 6)-linked glucan with α -(1 $\rightarrow$ 3) branches. The fructose moiety is released. The synthesised dextran forms a highly adhesive, water-insoluble polysaccharide matrix that mediates firm bacterial adherence, forming the dental plaque biofilm. The bacteria can then degrade this dextran via dextranases to produce glucose for glycolysis, leading to localised lactic acid production that demineralises tooth enamel and causes carious lesions.

Microbial Q3. What is the structural difference between native (high-acyl) and low-acyl gellan gum, and how does this affect their gelling properties?

Microbial A3. The native, **high-acyl (HA) gellan** gum carries two types of O-substituents on the first glucose residue of each tetrasaccharide repeat: a glyceryl group at C-2 and an acetyl group at C-6. These bulky substituents sterically hinder the tight packing and aggregation of the polymer's double helices. As a result, HA gellan forms **soft, elastic, and flexible gels**. **Low-acyl (LA) gellan** is produced by alkaline treatment to remove these acyl groups. The deacylated chains have no steric hindrance, allowing their double helices to aggregate extensively and tightly upon cooling with cations. This produces **hard, firm, and brittle gels** with high gel strength. This tunable gel texture via deacylation is a key industrial advantage of gellan gum.

Microbial Q4. Xanthan gum solutions display pseudoplastic (shear-thinning) behaviour. Explain this property on a molecular basis and give one practical advantage it provides.

Microbial A4. In static or low-shear conditions, the high-molecular-weight xanthan molecules exist as stiff, ordered double-helical structures. These stiff rods form an extensively entangled network throughout the solution, resulting in very high viscosity. When shear force is applied (e.g., pouring, shaking), the rigid molecules align in the direction of flow and the network of entanglements is progressively disrupted, causing a reversible, dramatic drop in viscosity. When the shear is removed, the molecules relax, re-entangle, and the high viscosity is restored. This shear-thinning (pseudoplasticity) is due to the rigid backbone and charged side chains. A practical advantage is in a **salad dressing**: the product pours smoothly from the bottle (high shear) but instantly re-thickens to cling evenly to the salad leaves (low shear), providing excellent adhesion and mouthfeel without dripping off.

Microbial Q5. Explain the role of NOD1 and NOD2 receptors in innate immunity, focusing on the specific peptidoglycan fragments they recognise.

Microbial A5. NOD1 and NOD2 are cytoplasmic pattern-recognition receptors (PRRs) of the innate immune system that detect intracellular bacteria by sensing specific fragments of peptidoglycan. **NOD1** primarily recognises **γ -D-glutamyl-meso-diaminopimelic acid (iE-DAP)**, a dipeptide fragment found predominantly in the peptidoglycan of Gram-negative bacteria (which use m-DAP in the third position of the stem peptide). **NOD2** recognises **muramyl dipeptide (MDP)**, which is MurNAc-L-Ala-D-isoGln, a fragment common to both Gram-positive and Gram-negative peptidoglycan. Upon binding their respective ligands, NOD1 and NOD2 oligomerise and recruit the adaptor protein RIPK2, activating the NF- κ B and MAPK signalling pathways, leading to the production of pro-inflammatory cytokines and antimicrobial peptides.

Microbial Q6. Why is sucrose specifically required for dextran synthesis by oral *Streptococcus mutans*, and not other sugars like glucose or lactose?

Microbial A6. The glucosyltransferases (Gtfs) of *Streptococcus mutans* are specific transglycosylases that use **sucrose** as their almost exclusive substrate because the reaction is thermodynamically driven by the high free energy of the sucrose glycosidic bond. The α -1, β -2 bond linking glucose to fructose in sucrose has a bond energy comparable to that of a nucleotide-sugar (like UDP-glucose). The Gtfs cleave this bond and transfer the glucose moiety to the growing dextran chain, releasing free fructose. Other sugars like glucose, fructose, or lactose do not possess this high-energy bond and cannot serve as direct glycosyl donors for polymer synthesis by Gtfs. This dependence on dietary sucrose is the direct molecular link between sugar consumption and the pathogenesis of dental caries.

Microbial Q7. Describe the "egg-box" model in the context of gellan gum gelation and compare it to the same model in pectin.

Microbial A7. The "egg-box" model describes the cation-mediated gelation of polyuronide chains. In **gellan gum**, the polymer forms double helices upon cooling. The carboxyl groups of the glucuronic acid residues in the repeating tetrasaccharide are located on the outer surface of these helices. Divalent cations like Ca^{2+} and Mg^{2+} electrostatically screen the negative charges and form salt bridges ("eggs in the box") between adjacent helices, causing them to aggregate into junction zones and form a three-dimensional gel network.

In **low-methoxyl (LM) pectin**, the model is analogous but involves individual chains, not helices. De-esterified blocks of α -1,4-galacturonic acid chains associate into two-fold buckled ribbons. Ca^{2+} ions fit into the cavities between these parallel chains, coordinating with the carboxyl and hydroxyl groups like eggs in a box. Both are cation-driven, but gellan requires a prior helix-coil transition, whereas pectin gelation is direct chain-chain association.

Microbial Q8. What is the function of the unusual D-amino acids (D-Ala, D-Glu) in the peptidoglycan stem peptide?

Microbial A8. The presence of D-amino acids, specifically **D-alanine** and **D-glutamic acid**, in the peptidoglycan stem peptide serves a crucial protective function: **resistance to proteases**. The vast majority of host and environmental proteases (e.g., trypsin, chymotrypsin) are specific for L-amino acid peptide bonds. By incorporating D-amino acids, the peptidoglycan stem peptide and its cross-links are rendered highly resistant to degradation by these common proteolytic enzymes. This ensures the structural integrity of the cell wall sacculus in protease-rich environments, such as during an animal infection. Additionally, the D-Ala-D-Ala terminus is a specific structural motif that is targeted by the transpeptidases

(PBPs) for cross-linking and uniquely recognised by glycopeptide antibiotics like vancomycin.

Microbial Q9. How does the side chain structure of xanthan gum contribute to its exceptional stability over a wide range of pH, temperature, and ionic strength?

Microbial A9. The exceptional stability of xanthan gum solutions arises from its unique molecular architecture. The **cellulosic β -1,4-glucan backbone** is inherently rigid and insoluble. However, the **charged trisaccharide side chains** (which include glucuronic acid) are attached at O-3 of every other glucose. These side chains are thought to fold back and align along the stiff backbone, forming a protective sheath via non-covalent interactions (hydrogen bonds and electrostatic interactions). This sheath sterically shields the vulnerable cellulosic backbone from chemical and enzymatic attack. Furthermore, the negative charges on the glucuronic acid and pyruvate groups maintain chain-chain electrostatic repulsion, preventing aggregation and precipitation. This combined shielding and charge stabilisation makes xanthan viscosity virtually unchanged from pH 2 to 12, up to high temperatures, and in high salt concentrations.

Microbial Q10. Compare dextran and gellan gum in terms of their biological origin, chemical structure, and gelation mechanism.

Microbial A10. **Dextran** is produced by lactic acid bacteria (e.g., *Leuconostoc*, *Streptococcus*) via secreted glucosyltransferases (Gtfs) acting on sucrose. It is a neutral homopolysaccharide with a backbone of α -1,6-linked D-glucose and α -1,3 branches. Dextran itself does not naturally form cohesive gels; it is water-soluble and acts as a viscous, adhesive biofilm matrix. To form hydrogels for chromatography (Sephadex), it must be chemically cross-linked. **Gellan gum** is produced by *Sphingomonas elodea* via an intracellular biosynthetic pathway and exported. It is an anionic heteropolysaccharide with a tetrasaccharide repeat of glucose, glucuronic acid, and rhamnose, carrying acyl substituents. Gellan is a truly physical gel; it undergoes a thermally reversible helix-coil transition, and the subsequent cation-mediated aggregation of these double helices forms a firm, three-dimensional network without chemical cross-linking. Dextran is a biological adhesive, gellan is a biological heat- and cation-responsive gelling agent.