COURSE CODE: **BCH 403**

COURSE TITLE: **Biosynthesis of Macromolecules**

NUMBER OF UNITS: 2 Units

COURSE DURATION: **Two hours per week**

COURSE DETAILS:

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COURSE CONTENT:

- ✓ Structure and functions of macro-molecules
- ✓ Storage and structural polysaccharides
- ✓ Muco polysaccharide, glycoproteins, bacterial cell walls synthesis of couples lipids
- ✓ Lipoproteins a nucleic acids

COURSE REQUIREMENTS:

This is a compulsory course for all final year Biochemistry students. In view of this, the students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

READING LIST:

1 Bohinski, R.C. 1983. Mordern concepts in Biochemistry. 4th Edition. Allyn and Bacon.

2 Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. 2003. Harper's Illustrated Biochemistry. 26th Edition. McGraw-Hill.

Polysaccharides (Glycans)

Most CHOs in nature exist as high-inert polymers called polysaccharides. They serve either a structural function or play an important role as a stored form of energy. All polysaccharides can be hydrolysed with an acid or enzymes to yield monosaccharide and one or simple monosaccharide derivatives. Those polysaccharides that upon hydrolysis yield only a single type of monosaccharide molecule are termed **Homopolysaccharides**. Those that yield a mixture of constituent monosaccharides and derived products on hydrolysis are called **Heterpolysaccharides.** D-glucose is the most prevalent monosaccharide unit in polysaccharides, but polysaccharides of D-mannose, D-fructose, D- and L-galactose, D-xylose and D-arabinose are also common. Monosaccharide derivatives commonly found as structural units of natural polysaccharide are D-glucosamine, D-galactosamine, D-glucuromic acid, N-acetymuramic acid and N-acetylneuraminic acid.

Structural and storage polysaccharides

When the supply of CHO to an organism exceeds the rate of utilization, the surplus CHOs are converted into storage polysaccharides which can be used later when demand exceeds supply formation of storage polysaccharides occurs typically in animals after feeding or in greed plant during photosynthesis. Microorganism, too can synthesis storage polysaccharides, especially if they are grown in CHO-rich media where the growth rate is restricted by limiting the course of an essential nutrient such as PO_4.

The advantages to the cell storing CHO as polysaccharides rather than monosaccharide lie in the physical properties of the molecules. Large quantities of free sugars would produce high osmotic pressure within the cell and would increase the uptake of H_2O. (Further explanation using diabetic patients). There is no such problem with the insoluble or osmotically inactive polysaccharides. Most cells, in fact, maintain a rather low internal concentration of free monosaccharide by converting them to PO_4s and other metabolites as soon as they enter the cell. This is important in maintaining a ″downhill″ concentration gradient for sugar transport into cells.

One of the requirements of storage polysaccharides is that they should be capable of rapid synthesis during periods of plentiful CHO supply and that they should be capable of rapid breakdown in response to energy requirements of the organism. To make room for rapid metabolism, most storage polysaccharides are deposited with the cell as roughly globular structures in which the polysaccharides chains have many loose ends available for enzyme action. In some plants, this is achieved by having short simple polysaccharides chains e.g. inulin. However, in nearly all animals, and in many plants and microorganism, this structure is achieved by having large polysaccharides molecules which are branched many times to give an open tree-like structure with many ends available to enzymes e.g. glycogen and starch.

The enzymes of polysaccharide synthesis and breakdown are often found loosely bound to the insoluble polysaccharides and this has been used as a basis for the isolation of such enzymes. It is probable that the association is of physiological importance in promoting rapid metabolism of the polysaccharides when required.

Glycogen: Glycogen is the main storage polysaccharides of animals and of some microorganism. It a polysaccharides of D-glucose, in which chains of glucose residues are joined together by α (1-4) glycosidic linkages. In addition, a smaller proportion (usually about 8-10%) of the total of α (1-6) linkage is present and it is these which form the branch points in the molecules.

The glycogen molecule is very large and may contain over a million glucose residues. Many α (1-4) linked chains are present. It is a more highly branched and more compacts molecule than amylopectin. The branching occurs at about every 8 to 12 glucose residues and they are the α (1-6) linkages. There is one reducing and per macromolecule of glycogen in which the terminal glucose unit is either unsubstituted at C-1 or linked to protein. All the other terminal glucose units are non-reducing with a free OH group at C-4 and with C-1 glycosidically linked to the

next glucose residue. The main enzymes of glycogen synthesis and breakdown in the cell (glycogen synthesis and phosphorylase) use these non-reducing chain ends as substrates.

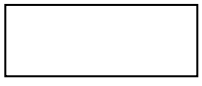
Glycogen is essentially abundant in the liver, where it may attain up to 10% of the wet. It is also present to about 1 to 2% in skeletal muscle, which are themselves chisters of smaller granules composed of single, highly branched molecules. Glycogen can be isolated from animal tissues by digesting them with hot KOH solutions. With this procedure, the non-reducing α (1-4) and α (1-6) linkages are stable, phosphorylase and glycogen synthesis are found to be associated with the glycogen particles after they have been isolated from tissues by careful procedures.

The most of glycogen molecules varies considerably, depending on the animal species, the type of tissue, and the physiological state of the animal. Even within a tissue, the glycogen molecules are polydisperse i.e. they have a wide range of waste. The glycogen of rat liver for e.g. has unit in the range 1-5x 10^8, whereas that from rat muscle has a rather low unit of about $5x10^6$ α is less polydisperse.

Glycogen is readily hydrolyzed by α- and β- amylases to yield glucose and maltose respectively. The action of β- amylase also yields a limit dextrin. Glycogen gives a red –violet colour with iodine.

Biosynthesis of Glycogen: The pathway begins with the conversion of G-6-P to G-1-P, a reaction catalyzed by phosphoglucomutase G-6-P $\rightleftharpoons$ G-1-P

The next step is the activation of G-1-P at the expense of a nucleoside $triPO_4$ to form the sugar nucleotide, the eventual donor of glucosyl residue to the glycogen growing chain. Bacteria use ADP- glucose whereas vertebrates use UDP –glucose as the glucosyl donor. In this activation step, an oxygen atom of the phosphoryl group of G-1-P attacks the α-P atom of the nucleoside $triPO_4$, displacing $pyroPO_4$. The reaction in animals is catalyzed by the enzyme G-1-P uridyltransferase



In the next step, the glucosyl group of UDP-glucose is transferred to the terminal glucose residue at the non-reducing end of an amylase chain to form an α (1-4) glycosidic linkage below C-1 of the added glucosyl residue and the 4-OH of the terminal glucose residue of the chain. This reaction is catalyzed by glycogen synthase.

$$\text{UDP –glucose} + (\text{glucose})_n \longrightarrow \text{UDP} + (\text{glucose})_{n+1}$$

The action of glycogen synthase is thus to bring about chain elongation by one unit at a time. Hence, repeated transfers of glucose residues from UDP –glucose will cause elongation of the outer chain of the glycogen molecule.

Glycogen synthase requires as a premier an α (1-4) –polyglucose chain having at least 4 glucose residues, to which it adds successive glucosyl groups it works best with long–chain glucose polymers such as amylase as primers. The requirement for a preformed polymer or ″primer” to act as an acceptor is a common feature of many enzymic reactions for synthesis of polysaccharides. There is also evidence that protein can act as a primer for glycogen synthesis and glycogen so formed would actually be a glycoprotein.

In addition to α (1-4) bonds glycogen contains α (1-6) bonds. Glycogen synthase cannot make these bonds found in the branch points of glycogen. The branching enzyme amylol (1, 4 -1, 6) trans-glycosylase is responsible for the formation of the α (1-6) linkages. This enzyme transfers a terminal oligosaccharide fragment of 6 or 7 glucosyl residues from the end of the main glycogen chain to the 6-OH group of a glucose residue somewhere in a glycogen chain. In the action of the branching enzyme, there is no net synthesis of glycosidic linkages, rather the enzyme acts by rearranging the he structure of the molecule and by forming the α (1-6) linkages at the expense of α (1-4) linkages.

Glycogen breakdown: There are 2 general mechanisms for the breakdown of both glycogen and starch. One mechanism involves hydrolysis of the polysaccharides to give glucose as the final product. This is characteristically an extracellular process, such as occurs in the alimentary canal during digestion of food. The free glucose is transportable, it can be absorbed, transported round the body in the blood and eventually taken up into cells. The 2nd mechanism for glycogen

breakdown involves phosphorolysis of the polysaccharide to give G-1-P as the major product. This happens intracellularly and the product is not transportable but is trapped within the cell. One advantage of phospho9rolysis is that the glycogen is converted directly to sugar PO4 without the intervention of hexokinase and consequence of ATP.

i) The intracellular process –Phosphorolysis, catalysis reaction

$$(\text{glucose})n + Pi \longrightarrow (\text{glucose})n\text{-}1 + \text{G-1-P}$$

Thus glucose units linked α (1-4) are round one at a time from the non-reducing ends of the glycogen molecule. This process continues until 4 glucose residues remain external to a α (1-6) linkage. Hence, action of phosphoroylase alone.

On glycogen gives a ″phosphorylase limit dextrin″. Further breakdown requires the presence of debranching enzyme which has 2 different catalytic functions in the same protein molecule. The 1st is a transferase which removes a short (optimum 3 residues) α (1-4) linked chain from attachments to an α (1-6) linked glucose and transfers it to an adjacent non-reducing end of the main chain. The 2nd enzymic activity, amylo (1-6) –α-D glucosidase, now hydrolyses the α (1-6) linkage to release free glucose. The debranched chain can acts as substrate for further phosphorylase action. The final products of the intracellular breakdown of glycogen are about 92% G-1-P from phosphorolysis of the α (1-4) linkages and about 8% glucose from hydrolysis of the α (1-6) linkages.

ii) The extracellular process: the extracellular digestion of glycogen and starch occurs in 2 stages. In the 1st stage, glycogen and starch are hydrolysed to give maltose and small unit oligosaccharides. This stage is catalyzed by α-amylse found in the saliva and pancreatic secretions of mammals α-amylase specifically hydrolyses α (1-4) linkages both in the outer and inner chains, beyond the branch points, thus causing rapid depolymerization of glycogen to large

oligosaccharides than occurs to give maltose and small oligosaccharides containing one or more α (1-6) linkages.

α-amylase cannot hydrolyse α (1-6) linkages of glycogen or the α (1-4) linkages of maltose. In the 2nd stage of digestion, maltose and the oligosaccharides are hydrolyzed to glucose by a mixture of glucosidases in the intestine complete hydrolysis to glucose is necessary before absorption can take place, since only monosaccharides are normally transported through animal cell membranes. The glocosidases (maltase, isomaltase etc) are associated with the brush border of the mammalian intestine. This is a region of the intestinal wall which has is a large surface area for absorption of products of digestion. The presence of the glucosidases in the brush border thus ensures that the final products of CHO digestion are released at the site where rapid absorption can occur.

Liver and Muscle Glycogen

Liver and muscle contain 95% of all the glycogen in the body of mammals. However, glycogen in these tissues functions rather differently. Glycogen of muscle (and many other tissues) serves as an enzyme reserve solely for the use of the particular cell in which it occurs. This the glycogen of working muscles decreases in amount when it is used to supply energy for contraction, but at the same time other non-working muscles in the body retain all of their glycogen. In contrast to muscle glycogen, liver glycogen serves as a major source of blood glucose. It is of crucial important for ensuring that glucose supply is maintained to other organs, especially to the brain. The metabolism of liver glycogen depends very much on the nutritionals state of the animal. Rapid synthesis occurs after CHO meal and the liver glycogen is later converted back to glucose in the post-absorptive state.

In keeping with its role in supply of blood glucose, liver (and kidney) contains an enzyme, glucose-6-phosphatase which is responsible for converting sugar PO_4s into free glucose. This enzyme is not found in muscle or in most other tissues. The overall pathways of glycogen synthesis and breakdown in liver are as show

Glycogen storage Diseases

These are rare diseases which arise as a result of a genetic defect in the ability of an individual to synthesis one or other of the enzymes of glycogen metabolism. These are summarized below

Glycogen Storage Diseases

Storage disease	**Defective enzyme**	**Tissue affected**	**Biochemical implications**
Von Grierke′s disease	Glucose-6 phosphatase	Liver	Glycogen breakdown to glucose is impaired. Liver glycogen high, blood glucose low during fasting. Liver does not respond to glycogen by releasing glucose
Andersen′s disease	Branching enzyme	Liver	Glycogen has abnormally long, unbranched chain
McArdle′s disease	Phosphorylase	Muscle	Impaired ability to exercise. Muscle glycogen high. No increase of lactate in blood during exercise
Cori′s disease	Debranching enzyme	Liver sometimes muscle	Glycogen is similar to phosphorykase limit dextrin, especially after fasting. Hypoglycaemia due to impaired ability to mobilize liver glycogen
Pompe′s disease	A-glucosidase of lysosomes	Most tissue	Glycogen accumulates in tissues
	Phosphorylase kinase	Liver	Impaired ability to use liver glycogen

Tarui′s disease	Phosphofructokinase	Muscle	Similar to McArdle′s disease

Starch:

Starch is the storage polysaccharide of most plants. It consists of a mixture of 2 similar polysaccharides of glucose –the unbranched amylose and the branched –chain amylopectin. In the starch of most plants, there is about 20-25% amylose and 75-80% amylopectin, but this ratio is not fixed and can vary in different plant species ant at different growth stages. The extremes of this variation are found in the starch from waxy varieties of maize (corn) which has virtually no amylase, and in the starch from a genetic variant of peas which has 70% amylose. Plants deposit starch in the fprm of granules, the shapes of which are characteristics of the plant species. Starch granules in ″long term″ storage tissues such as potato tubers are large (up to 100μm in diameter) and may constitute a large proportion of the total tissue weight. Here, the starch is laid down over a period of several months, and may then remain virtually unchanged until the following season, when it is used to provide substrates for new growth. The starch reserve of seeds is another long-term store. Seeds may remain dormant for years, but once germination starts, the starch is used within a few days.

Starch is also found in leaves, comprising about 1% of the dry weight of the tissue. Here it form a much more temporary store of CHO made by photosynthesis during daylight. At night, the starch is converted back to sugars and transported to non-photosynthetic tissues. In leaves, the granules are much smaller (about 1μm in diameter) than in long-term storage and are formed within the chloroplast where photosynthesis occurs.

When starch granules are heated in water, they swell, take up water, and gelatinize. After further heating, a colloidal solution of starch is formed. The amylose component can be separated from such solutions by addition of organic compounds such as n-butanol or thymol. The amylose

slowly precipitated out as a complex with the organic molecule leaving the amylopectin in solution.

Amylose

Amylose molecules consist of a 1000 or more glucose residues joined through α (1-4) linkage. Recent evidence suggest that there may also be a very small proportion of α (1-6) linkages. The near absence of branch points in the molecules allows them to take up a more regular conformation than is possible with the branched–chain structures of glycogen and amylopectin.

The conformation of amylose has been studied in complexes below amylase and iodine, or below amylase and organic molecules. Such amylose complexes are in a helical conformation, with 6 glucose residues per turn of the helix and the conformation of amylose is stabilized by the presence of the complexing molecules. However, it is probable that in aqueous solution, amylose exists in a much looser random structure. The complex of amylose with iodine has an intense blue colour and the ability to give such a colour is the basis of the iodine test for starch. The amylase–iodine complex consists of an amylose helix within which is included a linear polyiodine-iodide chain.

Amylopectin

The basic structure of amylopectin closely resembles that of glycogen. Both polymers have α (1-4) linked glucose chains branched by α (1-6) linkages. The most obvious difference below tham is that amylopectin has a lowered degree of branching than glycogen. In amylopectin, α (1-6) linkages comprise only about 4-5% of the total glycosidic linkages, giving an another chain length of 20-25 glucose residues, although variation in this does occur below different plant species. There is some evidence that the branching in amylopectin is not regular, but that there may be regions of dense branching. An asymmetrical cluster structure has been suggested for amylopectin

The branch points of amylopectin contribute significantly to the physical poperties of the molecule. Unlike amylase, the chains in amylopectin are interrupted by the branching and are unable to take up helical conformations of sufficient length to associate well with organic

solvents or with iodine. Thus amylase will complex ≅20% of its own with of iodine, whereas amylopectin complexes <1% iodine and gives intense than that of the amylose iodine complex.

Biosynthesis of Starch

Starch is synthesized by similar enzymes to those involved in glycogen synthesis in animals. The pathway also begins with the conversion of G-6-P to G-1-P. The next step, is the activation of G-1-P at the expense of a nucleotide triPO$_4$. In green plants, the activated form is ADP –glucose, formed by transfer of an AMP variety from ATP to the PO$_4$ of G-1-P by the enzyme ADP – glucose synthase

In the next reaction catalyzed by starch synthase, the oxygen on C-4 of the terminal glucoslyl residue of a starch chain attacks C-1 of a molecule of ADP-glucose, displacing ADP and adding the glucosyl unit to the polymeric starch molecule.

In non-photosynthetic tissues, the CHO substrate for starch synthesis may be supplied in the formed of sucrose. This can give starch by the following sequence of reactions.

Sucrose + UDP ⟷ UDP –GLc + Fructose (sucrose synthase)

UDP-Glc + PPi ⟷ Gk-1-P+UTP (G-1-P-uridylyl transferase)

Gluc-1-P+ATP ⟷ ADP-Glc+ PPi (G-1-P-adenylyl transferase)

ADP-Glc + (glucose)n ⟷ ADP + (glucose)n+1 (starch synthase

UTP + ADP ⟷ ATP + UTP (nucleoside diPO$_4$ kinase)

This pathway has been found to occur in developing pea and wheat seeds. It has the advantage that there is no overall utilization of ATP back, in effect, the potential energy of hydrolysis of the glycosidic linkage in sucrose is used in the synthesis of the glycosidic linkage of starch.

Formation of the branch points during amylopectin synthesis is catalyzed by the same type of transfer enzyme as for the branching enzyme of glycogen synthesis. The action of the plant enzyme (sometimes known as ″Q enzyme″) is such that it acts on longer chains and transfers longer sections than the corresponding enzyme from animals. It is not at all certain at present how the amylose and and amylopectin are formed as separate populations of molecules within the same starch granule. One possibilities is that separate enzyme complexes are responsible for synthesis of the 2 polysaccharides. According to this theory, a synthase –branching enzyme complex would synthesis amylopectin, whereas amylase would be synthesized by a synthase alone. Different nucleoside diPO_4. Sugar precursors may be used for synthesis of the 2 polysaccharides and, in support of this idea, is the observation that waxy cereal grains that are virtually devoid of amylase are unable to use UDP –glucose as a precursor. The starch granule grows by addition onto the outside and as a result, the molecules of product are probably arranged particularly to the surface.

Breakdown of Starch

Both phosphorylytic and hydrolytic mechanisms for the breakdown of starch mechanisms for the breakdown of starch are present in plants. Many plant tissues seem to contain both phosphorylases and amylases, and the relative physiological roles of the 2 processes is not as that clear as we have it for glycogen breakdown in animals. For e.g., plant leaves may contain sufficient activities of types of enzyme to account for the known rates of starch breakdown/ recent evidences however showed that of starch degrading enzyme in pea leaves chlorophyll only phosphorylase was present in the chlorophyll in sufficient to account for the rate starch breakdown, suggesting that in chloroplast, only a phosphorytic mechanism is involved in starch breakdown.

In some germinating seeds, the hydrolytic mechanism is most important. This may be related to the fact that in such seeds most of the starch reserves are stored outside the growing embryo and that the products of starch digestion have to be transported to the embryo.

The process of starch breakdown during germination has been particularly well studied in cereal seeds such as barley and rice. In barley for instance, the starchy endosperm is surrounded by a thin layer of cells called the a leurone layer, and it is these cells which bring about the

breakdown of the starch reserves of the endosperm. Two amylases are involved, β –amylase which is already present in an inactive form before germination begins and is converted to an active form during germination, whereas α –amylase is synthesized de novo by the aleurone cells after germination starts and secreted into the endosperm.

The α –amylase of plants has a similar action pattern to that of the α-amylase of animals i.e. it hydrolyses α (1-4) linkages of both exterior and interior chains in the amylopectin to give a mixture of maltose and small unit oligosaccharides. B-amylase is an exoenzyme which hydrolyses alternate α (1-4) linkages to give maltose. Unlike v-amylase, it attacks the external chains of amylopectin only, and cannot by-pass the branch points to attack the interior chains. The products of exhaustive hydrolysis of amylopectin by β-amylose are maltose and a high unit limit dextrin with 2 or 3 glucose residues external to the branch points. The α (1-6) linkages can be hydrolysed by another enzyme (amylopectin 6-gluconohydrolase, also know as limit dextrinase or R-enzyme) specific for this type of linkage. Unlike the debranching enzyme of animals, this enzyme does not possess transferase activity. Its action is to remove short chains of 2 or more glucose residues from the branch points of the substrate by hydrolyzing the α (1-6) linkages.

Other storage polysaccharides

Although glycogen and starch are by far the most common storage polysaccharides, in some species other types of polysaccharide are found to have storage. The presence of such polysaccharides is much more common in plants than in animals.

1 Dextrains: These are branched polysaccharides of D-glucose, but they differ from glycogen and starch in having backbone linkages other than α (1-4). Found as storage polysaccharides in yeast and bacteria, they vary in their branch points, which may be 1-2, 1-3, 1-4, or 1-6 in different species. Dextrans form highly viscous, slimy solutions.

2 Fructans: also called levans, are homo-polysaccharides composed D-glucose units. They consist of relatively short chains of fructose residue. They are found in many plants. The fructan ″inulin″ foubd in the bulbs of many plants (dehlias, Jerusalem artichokes) has fructofuranose residues joined in β (2-1) glycosidic linkages. Grasses (Gramineae family) also have fructans containing 2-6 linkages.

Inulin can be synthesized from sucrose by transfer reactions not directly involving nucleoside diPO_4 sugars. The synthesis starts by trans glycosdlation below 2 sucrose molecules catalyzed by sucrose-sucrose 1-fructosyltransferase.

Glc-Fru+ Glc-Fru $\longrightarrow$ Glc-Fru-Fru + Glc

In the next stage, a 2nd enzyme, fructosyl transferase adds on further fructose residues one at a time from the trisaccahride Glu-Fru-Fru until a relatively small polymer of about 30 hexose residues is completed

Glc-Fru-Fru +Glc-Fru-Fru $\longrightarrow$ Glc-Frut-Fru-Fru +Glc etc.

It is not known whether alternative mechanisms exist for the synthesis of inulin via nucleoside diPO4 sugars. Although UDP-fructose has been found in dealilia and Jerusalem artichoke tubers, the presence of enzumes converting this to polysaccharides has not been established.

3 Galactomannans found as storage polysaccharides in some fungi and yeast and in the endosperm of ″albuminous seeds from some plants of the leguminosae family. Typically, galactomannons have a main CHO chain of β (1-4) linked mannose residues onto which single galactose residues are joined through α (1-6) linkages. The frequency of substitution by galactose along the mannose chain varies according to the source of the polysaccharide.

2 functions have been suggested for the galactomannans, first, they serve as a reserve CHO for growth of the new plant. Thus during germination, hydrolytic enzymes are secreted by the a leurone layer into the endosperm, and the galactomannans are completely used within a few days. Secondly, they have the property of being able to retain water, and their presence may prevent complete dessication of seeds exposed to high temperatures.

4 Xyloglucan found in the seeds of several family

5 Laminaru (β (1-3) glucan found in some algae

6 Galactan –found in snails

7 Mannans –found in locust eggs

8 Paramylon (β (1-3) glucan –found in some protozoa (e.g. Euglena gracilis)

Structural polysaccharides

Most living cells are surrounded by extracellular structures which serve to protect and support them, for e.g. unicellular organisms such as bacteria have cell walls which may make up 20%-30% of the dry weights of the organisms. Higher plants also have cell walls; these cells and also help to support the whole plants. Animal cells do not have walls as such, but are supported and protected by skeletons, skins and connective tissues.

Nearly all of these extracellular structures are chemically complex and are mixtures of several different materials, each of which has a rather different function. In most structures, both proteins and CHO occur, and there may be specific interactions between the two. The CHOs found ibn these structures serve primarily as structural elements where they give shape, elasticity, or rigidity to plants and animal tissues as well s protection and support to unicellular organisms. Other components are also present apart from CHOs and proteins. For e.g. calcium salts occur in bones, teeth, and in crustacean exoskeletons. These structural elements are organized differently in individual cases. However, a common arrangement is one in which strong fibres of one material are supported in a matrix of a softer material non rods are embedded in cement to make reinforced concrete. Such an arrangement found in plant cell walls, where the fibres are of the polysaccharide cell, lose, in insect exoskeleton, where they are of another polysaccharide, chitin; and in animal connective tissues, in which the fibres are of the protein collagen.

Cell walls and coats are not only important in maintaining the structure of tissues, but also contain specific cell-cell recognition sites important in the morphogenesis of tissues and organs. They also contain other protective elements, such as the cell-surface antibodies of vertebrate tissues. For this reason, we shall examine the structural polysaccharides in the cortex of the molecular organization of cell walls and coats.

Bacterial cell walls

The interior of bacterial cells contains considerable quantities of dissolved substances (ions, metabolic intermediates etc). these exert a high osmotic pressure of up 20atm. These cell contents are separated from the external medium by the plasma membrane and since the medium is usually hypotonic relative to the cell interior, there is a strong tendency for the cell to take up

water osmotically. Without any restriction on this process, the cell would swell and burst. To prevent this, bacteria have a cell wall external to the plasma membrane which is capable of withstanding these osmotic forces.

1 They bear the antigenic determinants by which mammalian systems recognize them as ″foreign″ and are able to develop specific antibodies to counteract them.

2 The synthesis of the cell wall is the target for the action of a number of antibiotics, in particularly the penicillus

3 The mechanism of synthesis of the walls in an interesting biochemical system which has provided useful clues for the study of the synthesis of animal and plants extracellular structures.

Structure –Bacteria are classically divided into Gran-positive and Gran-negative organism, according to their reaction to the gran stain, an empirical procedure in which the cells are treated successively with the dye character violet and with iodine, then decolourized and treated with saframine. Although this property appears be rather arbitrary, it turns out that this classification divides bacteria into 2 groups which have a number of physiological and chemical differences. In particular, the cell wall structures of the 2 types are rather different. Both groups have in common the presence of a material known as peptidoglycan, which is the main structural components of all bacterial cell walls. However, the wall contain a number of additional components and these differ between the Gram-positive and Gram-negative bacteria. The Gram-negative bacteria are characterized by the presence of lipopolysaccharide which forms an outer membrane system external to the plasma membrane and the peptidoglycan. It is this which is presumed to prevent uptake of Gram stain. Gram-positive bacteria lack such a second membrane but usually possess a teichoic acid or teichnoronic acid. In general it is the additional components to the peptidoglycan which are on the outermost surface of the bacterium and which vary the antigenic determinants.

The general structure of peptidoglycan is the same for all bacteria, although there are detailed species specific differences in the identity of some of the amino acid components and in the type of peptide X-linking that occurs. The structure is built up as a network with CHO chains running in the other direction. Some, but not all, of the peptide chains are x-linked to the peptide chains of adjacent molecules, so that the 2-dimensional network is formed.

In Gram-positive bacteria, there are several layers of peptidoglycan with x-linking between layers, but in most Gram-negative walls, there is only one layer. The peptidoglycan completely surrounded the bacterial cell as a covalently bound network or ″bag –shaped macromolecules″ which has considerable resistance to outward pressure and thus protects the cell from osmotic lysis.

The basic recurring CHO unit in the peptidoglcycan structure is the nuropeptide. It is a disaccharide of the amino sugar N-acetyl-D-glucosamic and N-acetylmuranic acid (found only in bacteria) which are joined by β (1-4) linkages. The backbone may be regarded as a substituted chitin with D-lactic acid substituted on alternating residues. To the carboxyl group of the N-acetylmuraunic acid residuesof the backbone are attached tetra-peptide side chain, each containing L-ala, D-ala, D-glutamic acid or D-glutamie and either meso-diaminopinelic acid, L-L-lys, L-OHlys or oruithine depending on the bacterial species. For example in the Gram-positive bacterium *S. aureus*, the sequence of is L-ala-D-γ-glutamyl-L-lys-D-ala. In this tetrapeptide, the glutamyl residue is attached through its γ-COOH rather than its α-COOH. All the muramic acids are substituted in this way to form peptidoglycan strands. Because of the presence of D-amino acids the peptidoglycan structure of the bacterial cell wall is resistant to the action of peptide hydrolyzing enzymes, since these attack only peptides containing amino acids in the L-configuration.

The peptidoglycan strands are further linked to each other through their peptide side chains. The terminal D-ala residue of the side chain of one polysaccharide chain is joined covalently with the peptide side chain of an adjacent polysaccharide chain, either directly as in *E. coli* or through an interpeptide bridge as in *S.aureus*. in *S. aureus*, this bridge is a pentaglycine chain that extends from the terminal carboxyly group of the D-alamine residue of one tetrapeptide to the E-NH_2 group of the 3rd amino acid, L-lys in another tetrapeptide

In addition to the peptidoglycan framework bacterial cell walls contain a number of accessory polymers, which make up almost 50% of the weight of the wall. These accessory component differ from one species to another. There are 3 types of accessory polymers;

i) Teichoic acids

ii) Polysaccharides

iii) Polypeptides or proteins

The teichoic acids which make up from 20-40% of the dry weight of the cell walls of Gram-positive bacteria, are built up of chain of either ribitol PO_4 or glycerol PO_4. They usually carry additional species-specific components such as sugars or amino acids

structure of ribitol teichoic acid from cell wall of *S. aureus* n= 6-10

an important function of teichoic acids seem to be to concentrate cations, particularly Mg^{2+}, in the region immediately outside the plasma membrane. At ventral pH, the PO_4 groups of the teichoic acids carry negative charges which attract cations and act as ion exchangers on the cell surface. The important of this acidic materials is seem in bacterial grown on media containing small (limiting) amounts of PO_4. Such organisms use the available PO_4 for essential purposes such as nucleic acid synthesis, but stop making teichoic acids. Instead they make a new component called teichoinic acid residues but no PO_4. The carboxylate groups of the uronic acid residues give teichuronic acid the necessary negative charge so that it can functionally replace the teichoic acid.

The plasma membranes of Gran-positive bacteria also contain teichoic acids and these are always of the glycerol PO_4 type. They are enchored in the membrane by a lipid-linked tail, and are sometimes known as lipoteichoic acids. It has been proposed that the membrane lipoteichoic acis function to transport Mg^{2+} into the cell from the pool of ions bound to the wall teichoic acid. Lipoteicoic acids are not replaceable by teichuronic acids under conditions of PO_4 limitation.

The accessory polysaccharides in Gram-positive bacterial cell wall contain rhannose, glucose, galactose, or mannose (or their amines). These are external to the cell wall and depending on the species, they may be shiny or form hard, tough capsules. These do not appear to essential to the

life of the organisms, since mutants lacking these components are able to grow successfully. They may function to prevent desiccation, to act as a barrier to phage attack, and to provide a charged surface. Streptococcus mutants, which is partly responsible for plaque formations and densal decay, produces an extracellular 1,3-glucan which enables the bacteria to stick to teeth. Both teichoic acids and the polysaccharides of bacterial cell walls are antigenic.

The walls of Gram-negative cells such as *E. coli* are much more complex than those of Gram-positive cells. Their accessory components consist of polypeptides, lipoproteins and particularly a very complex lipopolysaccharide whose structure is just beginning to be understood. It is characterized by the presence of 2 unusual sugar in the molecule. The sugars, one a 7-carbon (haptose) called L-glycero-D-inamino heptose and the other, an 8-carbon sugar known as 2-keto-3-deoxyoctonate or octulosonic acid. The sugars form a trisaccharide backbone repeating unit consisting of 2 molecules of the heptose sugar and one molecule of the eight carbon sugar. To this trisaccharide backbone are attached oligosaccharide side chains and the fatty acid β-hydroxymyristic acid, which gives this complex structure its lipid character. The lipopolysaccharide forms an outer lipid membrane and contributes to the complex antigenic specificity of Gram-negative cells.

Synthesis of cell walls

The synthesis of the peptidoglycan component of the cell wall can be conveniently broken into 3 stages, which occur at different locations in the cell.

i) Synthesis of UDP-N-acetylmuramylpentapeptide inside the cell.

ii) polymerization of N-acetylglucosamine and N-acetylmuramyl-pentapeptide to form the linear peptidoglycan strans.

iii) x-linking of the peptidoglycan strands

Synthesis of UDP-N-acetylmuramylpentapeptide. The first stage in cell wall synthesis involves the synthesis of UDP-N-acetylmuramylpentapeptide. The first step involves the condensation of N-acetylglucosanmine 1-PO_4 with UTP to form UDP-N-acetylglucosamine. One formed, it then reacts with phosphenol pyruvate in a reaction catalyzed by a specific transferase to give the 3-enol-pyruvylether of UDP-N-acetylglucosamine

The pyruvyl group is then reduced to lactyl by an NADPH-linked reductase, thus forming the 3-0-D-lactylether of N-acetylglucosamine. Conversion of UDP-N-acetylmuranic acid to its pentapeptide form occurs by the sequential addition of the necessary amino acids. Each step requires ATP and a specific enzymes that ensures the addition of amino acids in the proper sequence. L-ala is added first, followed by D-glu, L-lys (attached by its and NH_2 group to the COOH group of the D-glu) and finally the dipeptide-D-alamyl-D-alamine as a unit

The latter dipeptide is formed by 2 enzymatic reactions: conversion of L=-ala to D-ala by a race mase, followed by the linking of the 2 ala residues in an ATP-requiring reaction to from D-ala-D-ala. All of these reactions take place in the cytoplasm of the bacterial cell.

An important function of the N-acetylmuramate is to act as a link between the CHO chain and the peptide, and this link is achieved by the formation of an amide bond between the COO^- group of the sugar and the NH_2 group of L-ala as the latter is added.

2 Formation of linear polymers of the peptidoglycan

This is the most complex stage in the synthesis of peptidoglycan and takes place in the plasma membrane. It may be divided into 5 steps (see diagram). The whole stage involves the polymerization of N-acetylglucosamine and N-acetylmuramyl-pentapeptide containing residues into linear peptidoglycan strands.

In step 1, the CHO pentapeptide is transferred from UDP to a lipid carrier in the plasma membrane. The carrier is a 55-carbon (C_{55}) isoprenyl alcohol known as undecaprenol phosphate, it has the structure

A pyroPO_4 linkage is formed with the lipid and UMP is released. N-acetylglucosamine is then transferred from UDP-N-acetylglucosamine in step 2 to the luipid intermediate this a typical transglycosylation reaction and UDP is released A β (1-4) glycosidic linkage is formed between the Glc NAc and the N-Mur NAc of the lipid intermediate. In step 3, 5 glyceric residues are sequentially added to the E NH_2 group of lysine. The glycine residues are activated by ester formation to a tRNA molecule.

In step 4, the disaccharide oligopeptide unit is transferred from the lipid intermediate to the growing peptidoglycan, and lipid PPi is generated. From here, we can see the dual function of the undecaprenol lipid. It not only activates the monomer for addition to the polymer, but it transports the monomer from the cytoplasmic side of the membrane to the extracellular side of the membrane, where cell wall assembly takes place. Little is known about the details of this transport process.

In the 5^{th} and final step, one PO_4 is hydrolyzed to regenerate the phospholipid, which then can react once again with UDP acetylmuramylpentapeptide and participate in another cycle, resulting in the addition of a new unit to the growing peptidoglycan strands. Dephosphorykation of the PPi form of the lipid is inhibited by bacitracin.

3 x-linking of the peptidoglycan strands

x-linking of the peptide chain of the peptidoglycan takes place outside the cell membrane by a transpeptidation reaction in which a new peptide bond is formed between the COOH group of the premultimate D-ala residue of one chain and the NH_2 group of the 3^{rd} amino acid in an adjacent chain

the reaction mechanism is such that the transpeptidase enzyme reacts with COOH end of the second to the last D-ala to form an acyl enzyme intermediate and the last D-ala is removed. As the peptide bond is formed, the enzyme is regenerated to start the reaction again.

The transpeptidation reaction takes place at the expense of the terminal D-ala-D-ala peptide bond, and can be viewed as a mechanism for peptide bond synthesis in the absence of ATP. This is very necessary in the environment outside the plasma membrane where there is otherwise no energy supply for the endergonic reaction of peptide bond synthesis. In addition to the transpeptidation reaction, some of the terminal D-ala is removed by a specific carboxyl peptidase, so that in the cell wall the peptide chains, chain contain 4 amino acids each, in contrast to the 5 of the precursors.

Synthesis of the other cell wall components take place according to the same general principles outlined for peptidoglycan assembly of precursor inside the cell, transport, though the membrane on a lipid carrier , and polymerization outside the membrane.

In Gram-positive bacteria where teichoic acids are present, the backbone structure (ribitol or glycerol PO^4) is synthesized from CDP ribitol or CDP-glycerol precursors by the action of teichoic acid synthase. Sugar side chains are then introduced into the teichoic acid chain from UDP derivatives.

Antibiotics which interfere with cell wall synthesis

To be of medical use, antibiotics not only have to interfere with some function essential to the life of bacteria, but must do this without affecting essential functions in the host. The best targets for antibiotics are therefore those in which the affected process in the bacterium differs markedly from that in mammals. Synthesis of cell wall peptidoglycan is much a process; it occurs by a unique reaction sequence, itr involves components (N-acetylmuramate, D-amino acids) not normally found in other organisms, and it is essential to the life of the bacterium.

The antibiotics that are known to interfere with peptidoglycan synthesis are only effective on growing cells (i.e. cells actively synthesizing cell wall) and kill the cells by osmotic lysis as a result of production of a weakened cell wall.

Antibiotic	**Analogue of**	**Site of action**
Phosphonomycin	PEP	Synthesis of UDP-N-acetylmuramate
D-cycloserine	D-alamine	Synthesis of D-ala and D-ala-D-ala
Penicillins and cephalosporins	D-ala-D-ala	Transpeptidase and carboxypeptidase
Bacitracin	-	Dephosphorylation of undecaprenol PPi
Vancomycin	-	Incorporation of lipid bound precursor into cell wall
Tunicamycin	N-acetylglucosamine	Transfer of N-acetylglucosamine onto precursor

2 of these antibiotics are very simple molecules. D-cycloserine is a structural analogue of D-ala and interferes with peptidoglycan synthesis by acting as a competitive inhibitor of 2 enzymes – the alamine racemase which is responsible for the synthesis of D-ala from its L-isomer, and D-alamine-D-alamine synthase. Phosphonomycin is also a simple molecule and is a structural analogue of PEP. It acts as an inhibitor of UDP-N-acetylmuramate synthesis by binding irreversibly to the enzymes in place of the substrate.

The penicillins and cephalosporins are a group of chemically similar antibiotics derived from amino acids. They bind to a number of different proteins in bacterial cells, but their most important effect seems to be inhibition of the transpeptidation reaction of cell wall x-linking. Penicillins also inhibit the carboxypeptidase which hydrolyses terminal D-ala residues from non-x-linked side chains. When some species of bacteria are treated with penicillins, a soluble peptidoglycan like products is formed which is defective in x-link and has pentapeptide side chains in place of the tetrapeptides of the normal product.

Plant cell walls

Plant cell must be able to withstand the large osmotic pressure difference between the extracellular and intracellular fluid compartments. Because of this, they require rigid cell walls to keep from swelling. In larger plants and trees, the cell walls not only must contribute physical strength or rigidity to stems, leaves and root tissues but must also be able to sustain large weights.

Most plant cell wall contain cellulose fibres embedded in matrix of other materials. The matrix has a rather variable composition, but the most common components are polysaccharides, water, glycol[protein and sometimes lignin. The proportions of these vary according to the species of plant and the stage of growth.

During development, 2 different phases of cell wall formation can be recognized.

1 Primary cell wall formation occurs during the main growth phase of the cell. The primary cell walls of different types of plant cell are rather similar in structure and are relatively thin. They consist of cellulose fibres in a relatively soft matrix with a higher water content. This wall is sufficiently plastic to allow some flow of components during wall expansion.

2 the secondary cell walls are formed after growth has ceased and while differentiation of the cell occurs. During this phase, considerable thickening of the walls with cellulose occurs, and lignin may also be incorporated. The wall formed at this stage is more specialized and is specific to the type of differentiated cell being formed.

Cellulose: It is the most abundant cell wall and structural polysaccharide in the plant would. It is also the major component of wood and thus of paper. Cotton is also cellulose to the core. Cellulose is also found in some lower invertebrates.

Cellulose contains D-glucopyranose residues linked through β (1-4) glycosidically. The chain are linear and may contain 2500 to 14000 glucose residues, thus making the unit of cellulose to vary (students to calculate most from information above). The glucose residues in the chain tend to take up a preferred conformation in which the ring oxygen of one

In the plants, cellulose molecules are assembled together in ″microfibrils″ which consist of bundles of 40 or more cellulose molecules arranged together in a regular pattern along the main axis of the complex. Adjacent chains are held together by 4-bonds between them. Although cellulose has a high affinity for water, it is the ability of cellulose molecules to give compact and tightyly packed aggregates which gives the microfibrils their high strength, unison,ibility and chemical inertness. In many fungal cell walls, chitin is the mains structural polysaccharide instead of cellulose.

On complete hydrolysis with strong acids cellulose yields only D-glucose, but partial hydrolysis yields the reducing disaccharide cellulose. When cellulose is exhaustively methylated and then hydrolyzed it yields only 2, 3, 6-tri-O-methylglucose (what does this tell us about cellulose?) all its glycosidic linkages are 1-4 and that there are no branch points.

Cellulose is not attacked by either α or β-amylase. In fact, enzymes capable of hydrolyzing the β (1-4) linkages on cellulose are not secreted in the digestive tract of most mammals, thus they cannot use cellulose for food. However, the ruminants e.g. cow are an exception. They can utilize cellulose as food since bacteria in the rumen form the enzyme cellulose, which hydrolyses cellulose to D-glucose.

Matrix polysaccharides: Higher plants contain a number of different matrix polysaccharide which cement the cellulose fibrils. These matrix polysaccharides are sometimes classified into 2 main groups- the hemi- cellulose and the pectic substances. The 2 classes differ in their solubility in various reagents. The hemi cellulose can be extracted from cell walls by alkaline solution whereas pectic substances are extracted by solutions of chelating agents or by acidic solutions. Hemicelluloses are not related structurally to cellulose but are polymers of neutral pentoses like D-xylose. Here, the xylose residues are linked in β (1-4) with side chains of arabinose and other sugars like mannose, galactose, glucose. Pectic substances are often uronic acid containing polysaccahrides of which the most common type is a β (1-4) linked D-galacturonan. The COOH groups of the uronic acids are esterified to a greater or lesser extent with CH_3 groups. Any non-esterified COOH groups are negatively charged and bind Ca or Mg ions in the cell wall. Pectin

substances may contain additional species specific sugars glycosidically linked to some of the uronic acid residues.

The pectic substances can form viscous sticky solutions and play an important roles in the middle lamellae between cells where they act as a cement to hold the cells together. It is these substances too, which are solubilized from fruits during characteristic gel texture.

Another component of plant cells wall is a complex glycoprotein sometimes referred to as extension. It is attached covalently to the cellulose fibrils and contains peptide chains with a high proportion of the amino acid hydroxyproline. In extension, arabinose containing oligosaccharides are glycosidically linked to the OH proline residues and in addition some galactose may be linked to serving residues in the protein. It is not certain what function extension plays in the wall. One suggestion is that it has a structural function along with matrix polysaccharides, and another suggestion is that it is involved in the synthesis of the cell wall.

The cell wall of higher plants can be compare to cases of reinforced concrete in which the cellulose fibrils correspond to the steel rods and the matrix material to the concrete. With this set up, the cell walls are capable of withstanding enormous weights and physical stress.

Wood contains anther polymeric substance lignin, which is a polymer of aromatic ROHs. Other polysaccharides serving as cell wall or structural components in plants include agar of seaweeds, which contains D- and L-galactose residues, some of which are esterified with H_2SO_4, alginic acid of algae and help which contains D-mannuronic acid unit; and gum Arabic, a vegetable gum, which contains D-galactose and D-glucouronic acid residues, as well as arabunose or rhamnose.

Synthesis of the plant cell wall

The general process for synthesis of plant cell walls resembles in some respects the synthesis of bacterial walls, synthesis of nucleoside diphosphate sugar precursors, within the cell, transport across a membrane, and polymerization outside the membrane. Additional complications exist in plants cells because the eukaryotic cell has a multiple membrane system and because the cellulose is assembled into microfibrils.

Synthesis of the matrix polysaccharides is believed to occur in the internal membrane systems of the cell, particularly the Golgi apparatus.

The synthesis of cellulose appears to be rather different from that of the matrix polysaccharide and probably takes place at the plasma membrane. The individual cellulose molecules have to be organized into microfibrils, and the most likely process to achieve this is one in which all of the molecules making up a microfibril are syntheses synchronously at a growing microfibril end. There is some uncertainty as to the nucleoiside diPO glucose precursor of cellulose. There is evidence that in some plants it is GDP-glucose, but for others it could be UDP-glucose. Recent, work on algae by Hop and his colleagues however indicate both nucleoside diPO4 sugars are needed for the synthesis of cellulose. These scheme is as shown Hopp *et al.* propose that dolichyl pyrophosphoryl glucose is the starting point for the synthesis of cellulose ″primer″ and that it is produced by the reaction of dolichyl PO4 and UDPGlc.

Transfer of 2 further glucosyl groups from UDPGlc occurs to yield a Dol PPGlc Glc Glc having β (1-4) linkages between the gla residues. Further transfers of glucosyl groups from UDPGlc occurs to form dolichyl phosphoryl glucose. This is then transferred to a protein to form a protein glucan complex which could then act as

The exoskeleton of arthropod

Many invertebrate have an exoskeleton which serves for protein as a skin and also acts as a frame to main the shape of the animal and to provide a firm base for muscle attachment. The best studied exoskeletons are those of the arthropods.

The insect exoskeleton consists of several different layers. The outermost layer of epicuticle contains waxes which make the animal impervious to water, and thus prevents desiccation. The main bulk of the cuticle is the exo- and endocuticle which are made up largely of a mixture of roughly equal amount of chitin and proteins. The outer layers of exocuticle are x-linked by sclerotization. The most important structural element in the exoskeleton of insect and crustaceans and in the cell walls of fungi is chitin. It is a linear β (1-4) polymer of N-acetyl-D-glucosamine.

Like cellulose, chitin molecules occur organized together in microfibrils consist of regular arrays of molecules held together by H-bonding bonding, and this arrangement gives the polymer its structural strength and resistance to chemical attack

The epidermal cell of insects secrete chitin in daily growth layers to give lamellae of chitin sandwiched between layers of protein matrix. The direction in which the microfibrils are aligned is changed from one day to the next, to give the cuticle a plywood –like structure with strength in all directions.

Biosynthesis of chitin