AMINO SUGARS

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Amino sugars are constituents of structures found in all tissues mostly on the surface of cells and in the spaces between them, forming the substance which binds cells together, membranes which envelop them and protective layers which cover them [1,2,3,4] Unlike most sugars which are obtained in the diet and are oxidized for energy, amino sugars are formed in the body from glucose and are committed entirely to the formation of structural components. The “glue” that holds tissues together is a meshwork of fibres of the protein collagen together with giant molecular complexes of proteoglycans, in which approximately half of the constituent molecules are amino sugars. These substances are in a constant state of formation, degradation, restructuring and recycling; the life of a constituent molecule in the complex is only a few days. Our diet may contain some amino sugars, but it is not an important source. They need to replace those lost in the steady turnover of tissue components, i.e.; their metabolism is met by synthesis starting from glucose (dextrose), our blood sugar. In skin, for example, about one fifth of the glucose is destined for amino sugar formation.

Synthesis of Amino Sugars

The process of synthesis begins with the conversion of glucose-6-phosphate to fructose-6-phosphate. This structural rearrangement of the molecule facilitates its interaction with an amino acid, glutamine. This is catalyzed by an enzyme which exchanges the amino group from the amide of glutamine for an oxygen on carbon 2 (C-2) of the sugar, forming glucosamine-6-phosphate. During these reactions, sugars are usually anchored by being bound to phosphate or through phosphate to a nucleotide. These interactions are important in directing the sugars and interacting with the enzymes which catalyze the changes in their structure. The first step is catalyzed by the enzyme glucosamine synthetase, which normally regulates the entire process, since it determines how much amino sugar will be made[5]

The second stop involves the addition of an acetyl group to the nitrogen atom which was added in the first reaction. The product is designated N-acetyl glucosamine-6-phosphate. If free glucosamine or N-acetyl glucosamine are present, they can be combined readily with phosphate and can enter into the synthetic processes. (For convenience, the phosphate or nucleotide will be assumed if not stated).

N-acetyl glucosamine (commonly referred to by its initials NAG) can be converted into two other amino sugars by the process of epimerization. This involves the shifting of an oxygen or other atom to the opposite side of the molecule. Although epimers have the same constituent atoms the location of any one of the opposite side has a profound effect upon the interaction of that molecule with other substances, accordingly each epimer has its own unique properties. An enzyme called C-4 epimerase shifts the oxygen and hydrogen on C-4 to the opposite location, indicated in the formula by writing it upward instead of downward as in glucosamine. The epimer is N-acetyl galactosamine. Another enzyme, C-2 epimerase, shifts the acetylated amino (acetamido) group, which was formed on C-2 in the first 2 steps, to the opposite side, as indicated, forming the epimer N-acetyl mannosamine. This sugar is extended by addition of another 3 carbon atoms to form N-acetyl neuraminic acid, also called sialic acid.

NAG, N-acetyl galactosamine and N-acetyl neuraminic acid (neuraminate), together with other sugars and sugar derivatives are found in 3 main classes of macromolecules (large molecules) where they are combined with, respectively, proteins, lipids (fats) or as chains made up entirely of sugars.

Incorporation of Amino Sugars into Macromolecules

Glycoproteins

Glycoproteins are proteins containing a chain of sugars, usually a dozen or so sugars, called oligosaccharide chain. This chain modifies the properties of the protein to which it is attached. The sugars are added to newly-synthesized protein in the endoplasmic reticulum and Golgi regions of the cell[6]. Some will be extruded from the cell as secreted products, enzymes, hormones, etc., while others will remain on the cell surface where the “antennae” of the oligosaccharide chains function in a specific manner; for example, as recognition and binding sites for hormones. Some glycoproteins with higher sugar content have special functions. Mucus contains a glycoprotein with a relatively high content of sugar (carbohydrate, abbreviated CHO). This gives it the property of forming a viscous solution.

The human body contains perhaps 100,000 different proteins, each comprised of an assortment of 20 or so amino acids. The arrangement of these into a peptide chain could be likened to the arrangement of letters of the alphabet into a phrase or sentence. Larger proteins might be compared to a whole paragraph, containing larger peptides or possibly several. Just as the sequence of letters conveys a very precise meaning, so too, the sequence of amino acids determines the unique properties of each protein, e.g. its role as an enzyme acting as a catalyst for a specific biochemical reaction. If one essential amino acid is missing, the protein cannot be formed: it would be like trying to type a paragraph on a typewriter with a key missing! This fact is well-known to nutritionists since ensuring an adequate assortment of essential amino acids is important in determining the nutritional value of proteins in the diet.

Amino sugars also confer specificity similar to that of amino acids. Many functions of proteins are dependent upon the structure of the oligosaccharide chain, including the example, their insertion on cell surfaces which govern the interaction with other cells and may determine the antigenic properties of the protein, and the lifespan of the protein in the circulation.

Glycolipids

Lipids with an oligosaccharide chain are cell membrane constituents in which the sugars are invariably located on the outer surface of the cell: An example is the glycolipid, called a ganglioside, which determines to what blood group we belong. In blood group A there is a molecule of N-acetyl galactosamine in the chain, while in group B this is galactosamine[7]. In group O, there is no sugar in that position in the chain. This illustrates the great specificity that can be determined by a single constituent amino sugar.

Hyaluronate and Glycosaminoglycans or Mucopolysaccharides

Hyaluronic acid (hyaluronate) is a polymer of alternating molecules of NAG and glucuronic acid. Glucuronic acid is derived from glucose by oxidation of C-6 to an acid carboxyl group. Hyaluronate forms long straight chains which can form a gel-like structure because of is great capacity to bind water.

This highly hydrated polymer occupies a space that is relatively very large as compared to other molecules of similar molecular weight.

Hyaluronate is usually found in association with other amino sugar molecules in large aggregates which it serves to link together. Glycosaminoglycans (GAG) or Mucopolysaccharides include chondroitins, keratans, dermatans and heparins. They are composed of NAG or N-acetyl galactosamine together with glucuronic acid, or its C-6 epimer, iduronic acid. The amino sugar and the acid alternate as in hyaluronate, but there are other constituent sugars as well. Also most of the amino sugars have sulfate groups on one or more positions which give the structure a strong negative charge and a hydrophilic nature, ie, a strong affinity for water, as in the case of hyaluronate. There are variations in the sugars present, in the length of the chains and other modifications in different GAG. The sugar chains are anchored to a protein called core protein, in which constituent amino acids serine, threonine or asparagine are attached to the oligosaccharides. The resulting structure is an extended protein "backbone" with oligosaccharide chains attached along its length. It has been described as a "bottlebrush" shape with the sugar chains attached like the bristles to a wire core which is the protein. The molecular weight is high, the whole structure is negatively charged, and binds a large amount of water.

Proteoglycans

Proteoglycans (PG) are giant molecular complexes which many molecules of GAG are attached through their core proteins to a long strand of hyaluronate. Whereas glycoproteins are mostly protein with a minor quantity of carbohydrate, the PG is mostly CHO, (90% or more). The whole structure has a molecular weight of millions, and is a major component of the extracellular space[2, 8]

Tissue Structures Formed by Macromolecules Containing Amino Sugars

The Interstitium

The space between cells in a tissue is occupied by a meshwork of fibres of the protein collagen. Collagen is the most abundant protein in the body making up a quarter of the total protein. There are several types: Type I, II and III vary somewhat in their structure. Interspersed in the network of collagen fibres are molecules of PG. The gel-like structure formed resists compression and limits the diffusion of molecules and movement of cells. This matrix binds cells together and regulates what passes among them. The enzyme hyaluronidase, once called "spreading factor" depolymerizes hyaluronate, breaking down the structure and allowing for freer movement e.g., of bacteria, in the interstitium[2, 8.] The type of collagen and the types of PG vary in different tissues, as well as the presence of other proteins. Each tissue has features of its own but the general pattern of the matrix is common. The constituent sugars and amino sugars, etc., which go into the structure of the matrix are synthesized in each tissue independently.

Basement Membranes

Basement membranes (BM) are structures composed of collagen and PG which surround blood vessels and tissues. The BM contain a special type of collagen, mostly type IV and a glycoprotein called laminin. Under electron microscopy, BM has 3 layers, the outer side bordering on the interstitium. A PG containing the GAG, heparin sulfate, is important in determining the permeability of the BM and thus what passes between blood and tissues[8].

Glycocalyx

The mucus membranes which line the digestive, respiratory and genitourinary tracts are covered by a thin coat of glycoprotein called the glycocalyx[3, 9]. Unlike mucus, which is secreted over the surface of the cells, the glycocalyx is an integral part of the cell membrane. Although it is very thin and can only be seen under the electron microscope, it is the ultimate barrier between the underlying cells of the intestinal mucosa, for example, and the contents of the intestine – digestive juices, bacteria, etc. It is also a filter through which all digested food must diffuse in order to be absorbed by the mucosal cells.

Some Tissue Components Containing Proteoglycans and Glycoproteins

Tendons and ligaments are rich in chondroitins

Cartilage contains these and keratan sulfates as well as a protein, elastin.

Synovial fluid, which occurs in joints: hips, knee, elbow, etc., is rich in hyaluronate which is not associated with protein but provides lubrication for the smooth movement of cartilagenous surfaces.

Mucus is a solution the main constituent of which is a glycoprotein with a relatively high CHO content. This gives the solution high viscosity which provides lubrication and protection for mucus membranes.

Skin contains chondroitins and hyaluronate.

The Eye has several structures containing PG ad GP. The aqueous humor is a solution of virtually only hyaluronate.

Blood Vessels: The Basement Membranes (BM) of all blood vessels contains collagen Type IV, a glycoprotein laminin and a heparan sulfate PG.

Heart Valves are rich in hyaluronate and chondroitins.

Sexual Organs are influenced by hormones which elicit profound morphological and functional changes many of which involve component PG. A striking example is the rooster's comb, which is largely hyaluronate whose synthesis is stimulated by androgens. The growth of a capon's comb is an old assay for male hormones.

The **sex skin** of the monkey is also rich in hyaluronate and stimulated by hormones.

The Placenta is an extension of the blood vessels of the fetus. Here, the BM of syncytial cells and fetal capillaries as well as intervening supporting material constitutes a major part of the barrier between the maternal and the fetal circulations [10] Recently, the synthesis of these components was reported by the stimulated by the steroid formed during pregnancy, 17 a-hyroxyprogesterone; the first indication of a specific functional role for the steroid[11]. Wharton's jelly in the umbilical cord is almost entirely hyaluronate, in fact, the best source for its preparation.

Chitin is a polymer of just NAG, analogous to cellulose, which is a polymer of glucose. Cellulose, the main constituent of plants, is the most abundant organic matter on earth; chitin, in shellfish and insects, is the second most abundant [12] In our bodies, chitin is found in fingernails and toenails which are almost entirely made of chitin.

Disorders of Tissues Involving PG [1, 2, 3, 4]

Earlier investigation was directed toward those tissues which are notably rich in PG such as cartilage, synovial fluid and mucus. Much of our present knowledge of the role of PG has been acquired only recently with the advent of appropriate techniques. Structures such as BM and interstitial PG are not easily seen in microscopic examination. Even with the powerful electron microscope, unless special procedures are used, many constituents are not visible and as a result, they have been ignored or overlooked and the importance of their role is just beginning to be recognized [8]

In Rheumatoid Arthritis and Osteoarthritis there is disturbances of ground substance formation, that is the interstitial matrix, and in synovial fluid. Current therapy includes anti-inflammatory drugs such as corticosteroids. Among other effects, corticosteroids induce polymerization of hyaluronate, but the primary biochemical defect remains to be established.

In Diabetes Mellitus there occur abnormalities of blood vessels as a complication of the disease. The BM of capillaries is thickened but is abnormal and does not function normally. This leads to poor circulation in limbs, to blindness (retinopathy) and to degeneration of nerves (neuropathy). These complications are known to be due to the formation of sorbitol from the accumulated glucose. The BM are notably deficient in neuraminate and undergo cycles of incomplete formation and breakdown [13] **The kidney** often shows lack of a structure called the glomerular polyanion which is rich in neuraminate and is a major factor regulating the excretion of large molecules such as proteins, which appear in the urine.

In Nephrotic Syndrome there is also a deficiency of the glomerular polyanion [14] but here it is not associated with other disturbances in CHO metabolism as in diabetes, and is restricted to the kidney.

Children with **Cystic Fibrosis** (CF) are usually born normal but develop obstruction in the digestive and respiratory tracts which leads to infection, inflammation and cyst formation which ends in destruction of organs [15]The cause of the obstruction is the thick mucus which is secreted in CF. They glycoprotein of the mucus in CF is heavily glycosylated, that is, has much more than the normal amount of CHO, and the oligosaccharide chains are longer. This leads to a solution which is excessively viscous [16]

In Inflammatory Bowel Disease (IBD) – Crohn's Disease and Ulcerative Colitis the synthesis of the glycocalyx which covers the mucosal cells had been reported to be normal [9, 17, 18] However, the turnover of cells in the intestinal tract, the highest in the body, is increased further in IBD [19] The incorporation of NAG was threefold greater than that of glucosamine in mucosal tissue from patients with IBD, indicating a demand that could be met with the acetylated sugar, the formation of which from glucosamine was not able to keep pace [20]

Intracranial hemorrhage (ICH) occurs in about half of all infants born 6 weeks or more prematurely, and is a major cause of neurological damage and disability [21] The cause of such hemorrhages is attributed to the weakness of blood capillaries due to deficiency of the supporting structures which rupture in the brain causing hemorrhages. Attempts have been made to prevent ICH with the corticosteroid hormone cortisol and with a hemostatic drug, Ethamyslate. Both agents were tried because they stabilize hyaluronate and could improve the integrity of capillaries. Cortisol is ineffective; Ethamsylate had some effect in less severe ICH. Neither agent stimulates the synthesis of hyaluronate and PG. The recent report that the steroid 17 α-hyroxyprogesterone stimulates synthesis of these substances in placenta [11] suggests that, as placenta is an extension of the fetal circulation, the decline in levels of this steroid at birth would remove such a stimulus. Most ICH develops in the first few days postnatally. Defective **Heart Valves** are also common in premature infants; these structures, too, are rich in hyaluronate and chondroitins.

Amino Sugars, Cell Proliferation and the Auto-Immune Theory

A widely-held theory of the cause of many diseases is that they result from the formation of antibodies to the body's own tissues, which then attack those tissues and cause damage. Such antibodies can frequently be found in the circulation and there is experimental evidence for their formation in a number of situations [22] Treatment of many conditions thought to be "auto-immune" in nature has been with agents which are known to suppress antibody formation, thus they are called "immunosuppressive agents". These include corticosteroids and other drugs such as methotrexate, cyclophosphamide, etc., which are also used in the treatment of cancer. Such treatment is very often successful, although there are serious side-effects. Cells of each tissue have their own characteristic life span and rate of replacement. Some, like certain nervous tissues are never replaced if damaged, although the cellular constituents do turn over. Cells which line the digestive tract have the highest rate, being replaced completely every 2 or 3 days. As a result of infection, injury or other disturbances, the lifespan of cells can be shortened, leading to an increased rate of turnover. This is a common accompaniment of many disorders of diverse origin. It is not easy to measure this process, though it has been done in some instances. An indirect measure of increased cell proliferation is the measurement of certain amino acid derivatives called polyamines which are formed in these conditions. The polyamines are measured for example, to detect the presence of a tumor somewhere in the body, but also give an indication of cell proliferation which is greater than normal.

Increased cell turnover could impose a strain on the biosynthetic capabilities of cells, resulting in inadequate formation of some essential components including the extracellular matrix. This would result in derangement of tissue architecture which would impair the integrity of the tissue and its functions. The leakage of proteins from damaged tissue could result in the formation of antibodies which in turn might react against the tissue. Whether this is an initial event or just part of the overall process is a debatable point, but some of the deficiencies caused might be amenable to treatment by replacement. For example, in the intestine in IBD the increased demand might be met by NAG. Corticosteroids and immunosuppressive drugs, since they inhibit cell division, might slow the rate of cell proliferation which then lessens the strain on the biochemical pathways in the cells. Consistent with this is the effect of nicotine in ameliorating symptoms in IBD [23] Nicotine is not an immunosuppressive agent but it is a vasoconstrictor, which reduces blood flow to the gut, which slows metabolism and cell growth. This might then allow the synthetic processes to keep pace with reduced demand. If some of the symptoms of a disease which have serious consequences can be dealt with by simply providing a needed substance, the use of drugs with serious side effects might be lessened or avoided.

There is much evidence that the intestinal tract with its large area for absorption is a major portal of entry for substances which cause immunological reactions, generally referred to as food allergies [25] The intestinal mucosa in such cases has been shown to be more permeable than normal, allowing offending substance to be absorbed which are normally excluded [26] Glycoproteins and proteoglycans play a key role in determining what passes between and into cells and thus in maintaining the entry of injurious agents.

The amino sugars which make up these various tissue constituents are normally made in the cells where they are required. Each tissue has its own enzymes for the necessary processes. Unlike the deficiency of an essential amino acid, which would affect all cells in the body, a deficient process involving amino sugars could be restricted to one or a few types of cells, for example the glomerular polyanion in the Nephrotic Syndrome or the glycocalyx in IBD. However, providing a supply of ready-made amino sugar could still provide the needed material to allow the synthetic processes to continue, since amino sugars are readily absorbed and utilized. Amino sugars are normal, physiological substances with no known undesirable properties. The one of choice is probably NAG, since it is neutral, is more stable and more soluble than glucosamine, and can be converted into other needed amino sugars. It circulates in the blood for several hours, very little is excreted and it is used exclusively for the formation of the substances described. There might, however, be instances where one of the derivatives is lacking due to inadequate function of one specific pathway of biosynthesis, in which case that particular amino sugar might be required. Modern research is gradually revealing the details of these processes which might underlie many derangements of tissues in diseased states [2, 3, 24]

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