Sulfate and Sulfation

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Summary

1. **Sulfate is essential for many biological processes.**
2. **Sulfate is needed for formation of proteins in joints; low levels of sulfate are found in plasma and synovial fluid from patients with rheumatoid arthritis.**
3. **Sulfate is needed to start the cascade of digestive enzymes released from the pancreas. Without proteases, lipases and amylases, food is not digested efficiently.**
4. **Sulfate is essential in forming the mucin proteins which line the gut walls. These have 2 main functions- they stop the gut contents from 'sticking' and they block transport of toxins from the gut to the bloodstream. Low plasma sulfate has been found in patients with irritable bowel disease.**
5. **Sulfate is necessary for formation of brain tissue. Before birth, the functional units of the brain, 'neurons', are laid down on a scaffolding network of sulfated carbohydrate chains. Reduced sulfation can lead to faulty neuronal connections and later dysfunction.**
6. **Sulfation is a major pathway in detoxifying drugs and environmental contaminants.**
7. **Sulfate is not easily absorbed across the gut wall. Recent research has shown that it can be absorbed across the skin. It is also formed in the body by oxidation of the aminoacids cysteine and methionine. However, this pathway is often sub-optimal and many people benefit from sulfate supplementation.**

Background

The process of sulfation involves adding on a sulfate residue, which has two negative charges, to a biological molecule. This leads to a number of 'knock-on' effects because the addition of a highly charged polar group can fundamentally alter the molecular configuration and properties. Sulfation can activate or inactivate a wide range of biological compounds and any aberration in the supply of sulfate can have potentially serious consequences.

Principally, sulfation is a major inactivation pathway for catecholamines such as the neurotransmitter dopamine, about 80% of which is sulfated in man (1). Usually, when chemical neurotransmitters are released in the central nervous system, they act at receptor proteins and are then inactivated by sulfation or by FAD-linked mono-oxygenases or alternatively are carried by transporter proteins back into the initiating neurone. Failure of a major pathway such as sulfation will lead to a neurotransmitter imbalance and this can have effects on behavior, mood and function, as can be seen in migraine where many patients have reduced sulfation capacity. The process of sulfation also affects the functioning of peptides and proteins. Mucin proteins, which line the gastrointestinal tract, are sulfated glyco-proteins which control adhesion/lubrication of gut contents and absorption of nutrients (2). They have long peptide backbones with repeating sub-units and also peptide side-chains, rather similar to a 'bottle-brush'. These amino acid sequences are glycosylated with a range of sugars and both these carbohydrate residues and the peptides themselves are sulfated. As the addition of sulfate residues imparts net negative charges, the proteins adopt an extended configuration to minimise their interactions, since the negative charges repel each other . Removal of the sulfate residues leads to a protein which has a more globular structure and provides less protection for the tissues from the intestinal contents. Reduced sulfation has been linked with gut dysfunction in irritable bowel disease (3) and lower levels of sulfation of the ileal mucins have recently been shown to occur in children with autism (4) and in irritable

bowel. This correlates with the finding that gut permeability is increased in a large percentage of autistic children (5). The effects of mucin modification can also be seen in the colonisation of the gut by microflora as sulfation increases resistance to colonisation by pathogenic bacteria (and viruses). It is interesting that *Helicobacter pylori*, which can colonise the stomach, only does so when it has produced a sulfatase enzyme to de-sulphate the gastric mucins (6). This reduced sulfation of mucin proteins may underlie the relatively common finding of *Candida* infections in the gut, since the slight negative charges on *Candida* cells would lead to their repulsion by the negatively charged sulfate groups on normal mucins. The reduction in mucin sulfation is probably an indirect cause of the alterations in gut flora which are often found in gastrointestinal inflammation.

Peptides can also be sulfated, usually on tyrosine residues,by the enzyme tyrosyl protein sulfotransferase (TPST); the gastric hormones gastrin and cholecystokinin are good examples of this pathway. Both are involved in the digestive process and both are activated by sulfation. In a complex cascade, gastrin is sulfated and, with hydrochloric acid from the stomach, causes release of cholecystokinin, which also requires sulfation. Together with peptide fragments released from gastric proteolysis (mediated by the hydrochloric acid), this acts with the peptide hormone secretin on pancreatic tissue to induce the secretion of a range of proteolytic enzymes and also amylase and lipases. Lower levels of pancreatic amylase alter the digestibility of starch-based foods and allow increased fermentation of pathogenic bacteria while the decreased pancreatic lipase activity promotes formation of foul-smelling fatty stools which contain undigested triglycerides. Without the sulfation process to trigger the release of pancreatic proteases such as trypsin and chymotrypsin, the complete digestion of proteins to their amino acid building blocks (proteolysis) cannot take place, so that peptides, rather than amino acids, are found in the gastrointestinal tract. As reduced sulphation of mucins may have made the gut more permeable, the stage is set to allow peptides to penetrate into the blood stream. Some peptides, particularly those derived from casein and gluten, have been found to be neuroactive with effects on the brain where they can act at opioid receptors, affecting behaviour, mood and responses to physical stimuli such as pain. This 'leaky gut' hypothesis therefore links with the opioid theory to explain why peptides from casein and gluten in particular seem to cause problems for some people. Although the blood-brain barrier is usually seen as being non-permeable to many compounds it may, like the gut, be 'leaky' in individuals with previous head injuries or who are living in stressful conditions. Several studies have reported the presence of brain-derived proteins and antibodies, such as those from myelin, within the peripheral circulation. If relatively large proteins can cross <u>from</u> the brain, it seems possible that peptides and proteins could potentially be transported <u>into</u> the brain, although the mechanisms involved are not known. Simple diffusion across 'leaky' gap junctions may be all that is necessary.

The supply of sulfate in man is rarely sufficient to allow synthesis of all the required sulfated biocomponents. There are two main sources of sulfate *in vivo*. Unlike other anions, such as chloride (Cl^-), which seem to be readily and easily absorbed, sulfate is carried into the body from the g.i. tract *via* a sodium ion-linked sulfate transporter (7). This is easily saturated, so that ingestion of many small divided doses of sulfate over a period of time leads to higher blood sulfate concentrations than when a single large dose is taken. Anaerobic bacteria can convert sulfate (SO_4^{2-}) into sulfide (S^{2-}); individuals with bowel disease may have a range of pathogenic species of bacteria in the gastrointestinal tract which readily carry out this reduction so any ingested sulfate may be reduced to sulfide and therefore become unavailable. Magnesium sulfate is readily absorbed across the skin so topical applications or bathing may be more successful in raising plasma sulfate levels. Sulfate is also produced *in vivo* by oxidation of methionine or cysteine, both sulfur - containing amino acids which are provided from dietary proteins, and this pathway probably provides ~80% of the sulfate required in man. The first stage in this process involves the enzyme cysteine dioxygenase (CDO); cysteine sulfinic acid is formed and undergoes fission to provide sulfite (SO_3^{2-}) ions which are then further oxidised to sulfate (SO_4^{2-}) ions by the enzyme

sulfite oxidase (SOX). As can be seen, if CDO or SOX have reduced activity, the provision of sulfate will also be decreased. The human CDO gene has 5 exons and 4 introns and is localised to chromosome 5 (5q22-23); it is similar to the rat gene and proteins from the 2 genes have about 90% homology. The gene has a Ptx-3 response element, with a range of cis-acting elements (expressed in the CNS exclusively in dopaminergic neurones). The CDO protein is found in heart, thyroid and kidney, as well as brain and the liver. There seem to be variations in the properties of CDO enzymes from different tissues which may reflect differences in degree of glycosylation. CDO is known to be polymorphic in human populations and there are sub-sets with lower activity (~ 30% of the population) or nul activity (~ 3% of the population) (8). The nul S-oxidisers are heavily over-represented in chronic disease states with an auto-immune component such as rheumatoid arthritis and primary biliary cirrhosis (9,10). Even within 'normal' populations there is a wide variation in the extent of conversion of dietary sulfur amino acids to sulfate. In experiments carried out over decades in this department in practical classes with medical students, it was found that increased levels of protein in the diet produced proportional increases in urinary sulfate excretion, as would be expected. However, about 30% of the students reached a plateau in sulfate excretion, despite increasing ingestion of protein, suggesting that some pathway or pathways had become saturated. Provision of sulfate for *in vivo* metabolism is therefore a rate-limiting step for many people.

In some conditions, large amounts of sulfate are lost from the body in urine; the kidney (as well as the gut) may be 'leaky', so that sulfate is not resorbed as would be expected. Sometimes this is linked with an increased excretion of protein/peptides in urine , suggesting that kidney function is impaired. There may, of course, be dysregulation of the sulfate excretion system. On the apical or brush border side of the cells of the renal tubules, the sodium-dependent NaSi-1 sulfate co-transporter removes sulfate from the filtered fluid and this is exchanged for other anions, usually bicarbonate so that sulfate is transported into the blood from the baso-lateral side (11). Another renal sulfate transporter, Sat-1, is a sulfate/bicarbonate/oxalate exchanger and has been found in the brain and is highly expressed in the cerebellum and hippocampus (12). This complicated process suggests that conservation of sulfate anions is critical for human function. It has however been suggested that the kidney transporters respond to the levels of sulfate in the gastrointestinal tract so that sulfated glycosaminoglycans(GAGs) lost from the intestine during chronic infection could release sulfate and so provide a signal to the renal transporter to excrete the anion (13). The sulfate transporters NaSi-1 and Sat-1 are both subject to regulation by a member of hormonal and dietary changes (14). Renal re-absorption of inorganic sulfate is increased in growth and development (15) and regulated by vitamin D, dietary sulfate, glucocorticoids and thyroid hormones (16), also metabolic acidosis (17) and non-steroidal anti-inflammatory drugs (NSAIDS) (18) and chronic potassium depletion (19). To add to a complex interaction, the renal sulfate transporter proteins are themselves sulfated and the brush border contains a matrix of sulfated GAGs, so reduced levels of sulfate *in vivo* could have the effect of altering the structure of the systems required to retain sulfate in the body. This would of course result in a spiral of increasing dysfunction and it is unclear which are the controlling factors. Further, most of the work on sulfate metabolism has been done with the rat and should be invoked with caution since this species uses different pathways from those seen in human beings.

Not only is there an impaired level of sulfate in many disease states, there is also often a corresponding lack of sulfotransferase activity. These are the enzymes which carry out sulfation of a wide range of substrates. They belong to a super-family which uses PAPS (3'-phosphoadenosine - 5'-phosphosulfate) as co-factor and are widely distributed throughout the body (20). This process is critical for life as a defect in PAPS synthesis is lethal in humans (21) reflecting the wide range of biosubstrates which are sulfated. Sulfotransferases with endogenous substrates are important in tissue development and mice with defects in the Golgi-membrane enzyme which sulfates

glycosaminoglycans are non-viable as these polysaccharides cannot be converted to the unique binding sites which are recognised as signals for growth (22, 23). Other mutant mice without the heparan sulphate 20-sulfotransferase gene die from defective kidney development (24) suggesting that the sulfate/sulfation axis is vital in kidney development and function. These enzymes with endogenous substrates are membrane-bound while those which sulfate signal molecules such as steroids, thyroid hormones and neurotransmitters are cytosolic (25). The same enzymes also carry out the second stage in metabolism of drugs and related compounds to give water-soluble derivatives which are more readily excreted. All sulfotransferases have common motifs, as they contain a five-stranded parallel β-sheet flanked by α-helices which contains both the PAPS binding site and also the centre of the catalytic site, structures which are highly conserved throughout evolution. The substrate-binding sites, however, are totally different in the membrane-bound and cytosolic enzymes (26). The major enzymes responsible for the sulfation of phenols and catecholamines (SULT1A1 and 1A3 respectively) have been mainly studied in blood platelets where activity is approximately co-regulated with other tissues in the body, such as the gut and brain. SULT1A1 and 1A3 have 93% amino acid sequence identity while a third more recently discovered isoform, SULT1A2, differs by only 3 residues from SULT1A1. This is, however, sufficient to give it slightly different properties with regard to substrate repertoire, inhibitor sensitivity and thermal stability. All three genes are located close together on Chromosome 16. SULT1A1 is present in high concentration in the liver, brain, blood platelets and many other tissues while SULT1A3 is also found in platelets and brain but tends to be concentrated in the gastrointestinal tract rather than the liver. Only limited information is available on the distribution of SULT1A2 but it has been detected in the liver (by RNA) and some bladder tumours. There is a wide variation in SULT1A1 activity in human populations, as much as 50-fold having been reported by one group of workers (27). The level of enzyme activity in platelets correlates with the level of protein as detected by immunoblotting. A number of different alleles for SULT1A1 have been found and an alloenzyme with His 213 is less active than one with arginine at the same site. A range of different SULT1A2 alleles are also known and are in a linkage disequilibrium with the SULT1A1 alleles. Polymorphisms do not appear to have been reported for SULT1A3 although investigations on the platelet enzyme activity in families suggest that they may exist, since there is a wide range of activity which is highly heritable (28).

Sulfotransferase activity is known to be altered in some dysfunctional states, for example most patients with migraine have low SULT1A1 and sometimes low SULT1A3 activity (29). They are therefore less able to sulfate dietary phenols and catecholamines in the g.i. tract and in the bloodstream *via* the platelets as the SULT enzyme is co-regulated in both tissues. Sulfation results in inactivation of amines and these individuals are susceptible to foods which contain substrates for the enzymes (cheese/tyramine, chocolate/phenylethylamine, bananas/serotonin). The increased blood levels of compounds with neurotransmitter activity are thought to 'trigger' migraine headaches in those who are already susceptible. It has been shown that individuals who are susceptible to migraine are metabolically unstable (with raised excitotoxic amino acid levels) so that very small changes in blood and brain catecholamine levels are sufficient to provoke a migraine attack. SULT1A1 and 1A3 can also be inhibited by flavonoids (30) and by foods containing these compounds which typically occur in fruit and vegetables (31). The incubation of cytosolic preparations from 35 different fruits and vegetables with platelet sulfotransferases produced a wide range of results . Some cytosols inhibited the sulfation of both test substrates although most appeared to more potently inhibit 4-nitrophenol sulfation than dopamine. Ingestion of citrus fruit, especially oranges, and red wine is often reported as being a migraine 'trigger' and the component flavonoids (naringin and resveratrol) are inhibitors of both SULT1A1 and SULT1A3. Further studies with purified flavonoids have shown that some can virtually abolish SULT1A1 activity at very low (less than 100nM) concentrations but at higher concentrations (over 1μM) they appear to act as substrates for SULT1A3. The inhibitory effect can be partially

overcome by the presence of magnesium ions. These not only appear to enhance enzyme activity, but also to decrease enzyme sensitivity to flavonoids like quercetin by a factor of about five.
Other sulfotransferases can also be affected. The enzyme tyrosylproteinsulfotransferase (TPST) is membrane-bound and found in most tissues of the body, including the platelet and the gastrointestinal tract, where it is co-regulated (32). The TPST enzyme is a 50-54 kDa integral membrane glycoprotein of the trans-Golgi network. At least 2 forms are known, TPST-1 and TPST-2. The latter is a type II transmembrane protein of 377 amino acid residues, encoded by m-RNA originating from seven exons of a gene located on chromosome 22. A 304- residue segment in the terminal domain of TPST-2 has 75% amino acid homology to the corresponding segment of TPST-1 including conservation of the residues involved in binding PAPS. The enzyme and requires the presence of acidic residues on the amino-terminal side of the target tyrosine (33). The TPST family is highly conserved from the evolutionary standpoint, presumably because the post-translation modification of tyrosine residues in peptides and proteins has been required for millennia. A number of substrates are known and these include the leukocyte adhesion molecule P-Selectin glycoprotein ligand 1 (PSGL-1) which is required for binding to P-selectin on activated endothelium and IgA, IgM, IgCT and complement C4 (34). TPST is the enzyme responsible for sulfation of gastrin and cholecystokinin as well as the sulfation of proto-mucin monomers. Sulfated cholecystokinin (CS) has receptors in the brain as well as the gut and is required for release of the peptide hormone oxytocin. Vitamin B6 (pyridoxal phosphate) has been reported to inhibit both SULT1A1 and TPST. However, studies in this laboratory have shown that, as with quercetin, this inhibition can be blocked by magnesium ions and that a ratio of at least 1:1 magnesium:B6 will restore enzyme activity.

Carbohydrates are also substrates for sulfation, particularly the polysaccharide chains attached to glycosaminoglycans (GAGs). Here, there are specific patterns of sulfation which control a range of functions and vary by cell type. These sulfated GAGs are involved in G-protein signalling, calcium signalling, gap junctions (cell-cell signalling) and in differentiation and growth (35). As the enzymes involved in carbohydrate sulfation have relatively high Michaelis constants (compared with SULT 1A1/2, for example), low levels of sulfate will affect this pathway preferentially. Because sulfated polysaccharides and GAGs are so important in the development of the foetal and neonatal brain, any alteration in their structure may have deleterious effects on their function. Sulfate transport across the placenta increases dramatically around the time of birth when most of the glial cells are being formed and these increased levels of sulfate are associated with formation of astrocytes and oligodendrocytes from progenitor cells (36). It is of interest that children have higher levels of plasma sulfate than adults (0.47 nmol/l at birth decreasing to 0.33 nmol/l at 36 months; adults levels are around 0.27 nmol/l although there can be a wide range). This relatively high level of sulfate, as compared with the adult state and with, for instance, laboratory rats, may be associated with a requirement for sulfate patterning of carbohydrates in neuronal development. Recent studies on perineuronal nets have found these structures coating the proximal dendrites of certain neurons including those in 5 areas of the brain involved in autism (37). It seems probable that the nets play a role in signal modulation of the GABA-ergic neurons; they contain chondroitin sulfate and so are distinctive from other structures. In rat studies, these perineural nets have been found in the thalamus, which controls sensory integration and emotions and also in the hypothalamus, controlling hormonal and autonomic regulation neurons. Neurones in the human cerebral cortex which are ensheathed by perineuronal nets rarely undergo cytoskeletal changes in Alzheimer's disease, suggesting a neuro- protective effect of extracellular matrix components (38). Other proteoglycans with sulphated GAG chains also have a role in brain development, particularly the syndecan family which regulates the maturation of dendritic spines and also glypican which is expressed during axon growth. Neurocan is a chondroitin sulfate proteoglycan of the lectican family, mainly expressed during modeling and remodeling stages in the CNS. It binds to a wide range of cell surface molecules and to structural extracellular matrix components and has been

suggested as being responsible for the 'fine-tuning' of neural networks as it modulates cell-binding and neurite outgrowth (39).

Sulfation of proteins and carbohydrate chains is also important for the function of joints in the body. A special protein/carbohydrate complex exists to allow the surfaces of the different bones in eg wrist, knee and ankle to slide over one another as the joint is used. This slippery lining to the joints is called the 'synovium'; the surfaces of the bones are held slightly apart by the lubrication of synovial fluid. Rather like the mucin proteins in the gut walls, the synovium is heavily sulfated on proteins and associated GAGs to give a structure which is both very resistant to breakdown in use and also able to protect the bones in the joint from rubbing against each other and causing damage. Patients with rheumatoid arthritis have been shown to have very low levels of sulfate in plasma, synovial fluid and also in the synovium proteins (40) and this loss of sulfate is linked with the disease progression, showing that sulfation is vital for joint function.

Conclusion

As more research is done, the importance of the sulfate supply becomes more apparent, since sulfation plays such a major part in so many biochemical processes while sulfate generation in the body is often sub-optimal.

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