SULPHOCONJUGATION AND SULPHOHYDROLYSIS

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CONTENTS

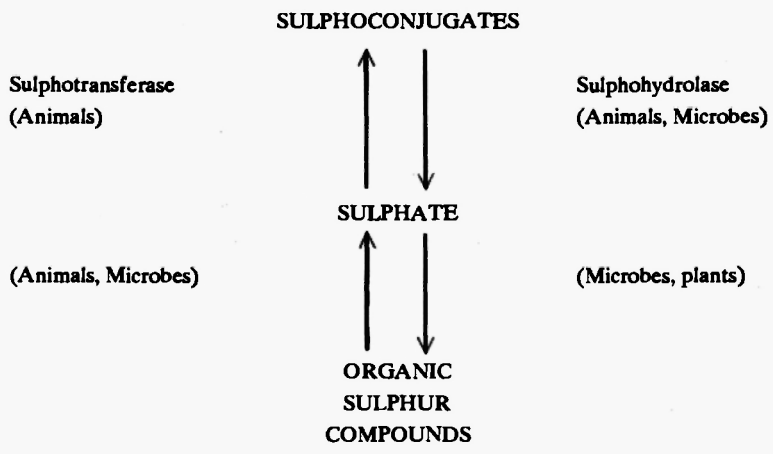
SUMMARY

The formation of sulphoconjugates is a ubiquitous phenomenon and the addition of the sulphate moiety to a variety of endogenous and exogenous molecules dramatically alters their physico-chemical properties and also their biological functions. Large numbers of different types of sulphoconjugate exist and their formation is catalysed by the versatile sulphotransferases. An equally versatile family of enzymes, the sulphohydrolases exist that are capable of accomplishing the reverse reaction. This paper comprises an appraisal of sulphoconjugation and sulphohydrolysis in the metabolism of xenobiotics and addresses the wider issues of sulphur availability and the interplay between mammalian and microbial enzyme systems in the sulphate cycle.

I. INTRODUCTION

In this review we seek to explain the current state of the art and provide a rationale for the integration of sulphoconjugation and sulphohydrolysis in relation to xenobiochemistry. There are excellent authoritative reviews on the individual areas and here, references are limited to these and the most cogent recent papers.

THE SULPHATE CYCLE



The history of research into the biological significance of sulphoconjugates and the enzymes responsible for their synthesis and hydrolysis is littered with misconceptions and misunderstandings /1,2,3,4,5,6/. There are two prime reasons for this. Firstly, some confusion persists about the role of the sulphate moiety in endogenous sulphate conjugates and secondly, the confusion was compounded by considerations of sulphoconjugation only as a detoxication device. The picture that has emerged is conceptually simple for Nature utilizes sulphoconjugation in several distinct ways: the sulphation of macromolecules such as glycosaminoglycans, of important endogenous relatively small molecules such as steroids, and of xenobiotics. In all cases, the addition of the sulphate moiety has a profound influence on the physico-chemical characteristics of the acceptor molecules and on their biological activities. Although this much is clear, much remains to be discovered about the significance of sulphate addition to both small and large molecules.

For many years, little interest was shown in sulphoconjugation and research on this topic was regarded by many as esoteric. There was a failure to appreciate that sulphoconjugates are not necessarily inert end-products of metabolism with "no physiological significance" but that some of them have essential metabolic and structural roles. For example, they have been shown to be metabolic intermediates and vital components of connective tissue. Their formation and hydrolysis are catalysed by large families of enzymes. The versatility of the sulphotransferases is such that there is enzyme capability for transferring sulphate to almost any type of acceptor. Similarly, there is an equally versatile family of hydrolytic enzymes, the sulphohydrolases.

II. THE ENZYME SYSTEMS OF SULPHOCONJUGATION AND SULPHOHYDROLYSIS

2.1 Sulphotransferases

The question now arises as to what roles the sulphotransferase enzymes play in xenobiochemistry. A simplistic view with considerable merit is that sulphoconjugation has much in common with glucuronidation in that it converts lipophilic and potentially toxic compounds

into hydrophilic molecules that are denied entry to cells, are pharmacologically immobilised and readily excreted. In short, sulphoconjugation is an effective, versatile, protective device which has evolved as a means of detoxifying putative toxins present in the environment. This view is undeniably supported by the fact that sulphotransferases with broad substrate specificities are present in tissues regarded as the first line of toxic challenge, viz the gut, liver and lung /7,8/.

The formation of sulphoconjugates is shown in the following general equation.

$$ROH + PAPS \longrightarrow ROS(=O)(=O)O^- + PAP$$

Invariably the sulphate donor appears to be PAPS (phosphoadenosyl phosphosulphate) but there is a wide diversity in the nature of the R group. Sulphoconjugates are known in which the sulphur atom from PAPS is attached to an O, N, S or C present in the acceptor molecule. However, *O*-sulphates arising from the sulphation of hydroxyl groups predominate. Hydroxyl groups occur in a variety of chemical environments but conjugation of the phenolic hydroxyl group is particularly common and many phenolic compounds produced during the normal course of metabolism and those presented exogenously are excreted as sulphoconjugates. Although there are well-documented examples of sulphoconjugates in which the sulphur atom is attached to nitrogen, they are much less common than those in which oxygen is involved. Similarly, conjugates involving S and C are known but rare.

The sulphotransferases as a group have proved to be a major challenge to the enzymologist /9,10,11/, being both numerous and difficult to handle. Early studies on their apparent specificity using crude tissue preparations suggested that a limited number of enzymes existed with fairly broad group specificity but different subcellular localizations.

Low molecular weight metabolites and most xenobiotics were seemingly sulphated by soluble cytosolic enzymes showing specificity for phenols, aliphatic alcohols or their derivatives whereas sulpho-

transferases for polymeric carbohydrates of connective tissues and also certain glycoproteins were located in the endoplasmic reticulum or Golgi fractions. More recent studies have revealed that a multiplicity of enzymes may be present within these groups and the cytosol in particular has yielded a large family of iso-enzymes. The complexity of the enzyme pattern, particularly with respect to subcellular localization, has prompted questions about the sources of sulphate available to the membranous and cytosolic sulphotransferases and indeed these appear to be different /12/. The metabolic fates of xenobiotics with respect to sulphation therefore seem likely to be determined by the facility with which a particular compound gains access to a sulphotransferase which in turn will use it as an acceptor.

2.2 Sulphohydrolases

The reverse of sulphoconjugation, viz. sulphohydrolysis, is a process seemingly present in all life forms. The range of specificities encountered in the sulphohydrolase group of enzymes is as extensive as the variety of sulphoconjugates and the list continues to grow. The advent of improved assay procedures and particularly the realisation that sulphoconjugates were not just inert end products of metabolism, kindled an interest in the enzymes which has revealed a complexity far beyond expectations. Studies on the phylogenic distribution of the various types of sulphohydrolases have revealed that in the lower forms of life, although many of the enzymes are constitutive, many more can be induced by a variety of sulphate esters. One must therefore conclude that there is a long-standing ability of micro-organisms to co-exist with or utilize naturally-occurring organic sulphates. Many more enzymes and substrates undoubtedly remain to be discovered and the reader is referred to the recent comprehensive review of this subject /13/.

The main thrust in elucidating the nature and physiological significance of the sulphohydrolases has come from studies of the enzymes of mammalian origin. Progress in this direction has been somewhat hampered by the use of convenient synthetic assay substrates only remotely related to the natural substrates and a preoccupation with their intriguing kinetic properties. A better understanding of at least the so called arylsulphatases has nevertheless

emerged. A breakthrough occurred with the discovery that deficiencies of the enzymes are responsible for a number of different inherited metabolic diseases /14/. A chance observation resulted in the important finding that the substrates for the soluble lysosomal arylsulphatases A and B were sulphated carbohydrate residues of connective tissue macromolecules rather than simple arylsulphates as used for their assay. Subsequently, the remaining enzyme arylsulphatase C was identified as a steroid sulphate sulphohydrolase and although probably the nearest to a true arylsulphatase, it now seems likely that it can hydrolyse steroid alkyl sulphates as well /15/. The revelation that these enzymes possess a rather greater breadth of specificity than hitherto suspected, necessitates a revision of the classification of the sulphatase enzymes into alkyl-, aryl- glyco- etc. Furthermore, the classification into Type I or II according to their enzymological properties and subcellular location which has been a useful basis for comparative studies, now needs to be revised. It should be remembered that sulphate is also liberated enzymically from compounds in which the linkage is not C-O-S but either P-O-S, N-O-S, or simply N-S. Although the enzymes involved are not strictly sulphohydrolases their activities are relevant to xenobiotic metabolism because some are involved in mammalian tissues, in the degradation of PAPS /13/.

The physiological roles of the mammalian intracellular sulphatases are therefore no longer quite so mysterious. The soluble lysosomal enzymes which are acid hydrolases are involved, together with a number of other glycosulphatases and depolymerases, in the sequential degradation of connective tissues and other structural components and so regulate the composition of the cells. This is a function characteristic of lysosomal acid hydrolases generally. The steroid sulphatase on the other hand is a firmly bound insoluble component of the endoplasmic reticulum and possibly also the nuclear membrane and is optimally active near neutral pH. We can visualise a role for the enzyme in the interconversion of a multiplicity of steroid sulphoconjugates and this in turn may also be related to steroid transport or even the regulation of steroid hormone action.

There may therefore be a tendency to assume that the situation is quite clear but a closer examination of the topic will reveal that a number of challenging questions particularly with respect to the steroid sulphohydrolase remain to be answered.

(a) What are the various processes regulated by the enzyme?
(b) Are these functions confined specifically to certain tissues or organs, for example what is the role of the enzyme in brain relative to that in say liver or kidney?
(c) What is the significance of the enzyme being localised on the cytoplasmic side of the endoplasmic reticulum membrane?
(d) What is the role of the enzyme in the endoplasmic reticulum compared with that in the nuclear membrane?

Answers to these and many other questions will be valuable in interpreting the metabolic pathways taken by xenobiotics as well as endogenous cell metabolites /15,16/.

III. SULPHATE PATHWAYS IN THE LIVER

Many cell types in many species are known to possess both sulphotransferase and sulphohydrolase activities. From the experiments performed this far, it is impossible to deduce how the activities of the enzymes relate to each other and to xenobiotic metabolism. For instance, the sulphotransferase activity *in vivo* and in cell preparations has been studied by measuring the rate of sulphoconjugate formation using suitably labelled substrates. Sulphohydrolase activity has been monitored by the release of ^{35}S-labelled inorganic sulphate from ^{35}S-labelled sulphate esters. Neither of these approaches has been particularly instructive for measuring the intracellular flux of components through the sulphation/desulphation pathways. Although the operation of the enzymes is readily detectable, it has not been possible to evaluate the effect of the interplay of sulphation and desulphation *in vivo* in relation to foreign compound metabolism. However, the complexities of these enzymes and others, for example, the glucuronyl transferases should not be under-estimated as factors influencing the toxicity and pharmacological activity of xenobiotics.

Even for relatively simple monohydroxylated compounds that undergo sulphation or glucuronidation in the liver, the intracellular flux and the subsequent partitioning of the conjugated metabolites between blood and bile is quite unpredictable and variable from one species to another /17/. Moreover the appearance of glucuronides in the bile or blood does not totally exclude the possibility that some intermediary sulphation has taken place. Since both the glucuronyl-

transferases and sulphohydrolases are located at the smooth endoplasmic reticulum this is a clear possibility. Whilst intermediary sulphation might be of little interest with respect to the end products of xenobiotic metabolism, it could be vital to the pharmacology/ toxicology of endogenous and exogenous sulpho-compounds with, for example, hormone-like activities.

Very little attention has been directed towards the possibility of sequential sulphation-desulphation followed by reconjugation with either sulphate or glucuronic acid (or both). Indirect evidence that this might occur in the liver of some species is provided by comparing the fates of ^{35}S-labelled sulphate esters of oestrone and diethylstilboestrol with the fates of ^{14}C-labelled oestrone and diethylstilboestrol /see ref. 17/. In liver perfusion experiments (rat and guinea pig) given 5 mol of ^{14}C-oestrone, about 0.13 mol appears in the bile as oestrogen sulphates (almost entirely oestrone sulphate in the guinea pig) and about 0.5 mol of oestrone sulphoglucuronide in the rat. However, when oestrone ^{35}S-sulphate is administered to rat livers it is rapidly hydrolysed at approx. 50n mol g^{-1}.min^{-1} and only trace amounts of oestrone ^{35}S-sulphate are detectable in bile /18/. Similarly, when diethylstilboestrol ^{35}S-disulphate is added to the perfused rat liver it undergoes extensive desulphation with very little sulphate ester in the bile. By contrast, when stilboestrol is added to perfusate at least two sulphoconjugates are excreted in bile (unpublished work). In short, data with esters of both oestrone and diethylstilboestrol suggest that hepatic desulphation and release of inorganic ^{35}S-sulphate is followed by some resulphation with sulphate of relatively low specific radioactivity. Moreover, when double conjugates (sulphoglucuronides) of oestrone are formed in the liver it has been suggested that sulphation is the first "detoxication" pathway /18/.

IV. SULPHUR STATUS

Animal sulphur status, the production and availability of sulphate are central to an appreciation of the occurrence and significance of sulphoconjugation /see 19,20/. Conceptually, the considerations are simple and theoretically the necessary information is not difficult to obtain. Nevertheless, in spite of considerable activity in sulphur and sulphate research, many questions, fundamental to an understanding

of the main issues remain unanswered and surprisingly little progress has been made in providing a co-ordinated fabric of information central to an appreciation of the status of sulphoconjugation. The reasons for this are complex but may stem from a failure to appreciate the biochemical significance of sulphoconjugates in general. Certainly the significance of sulphation of macromolecules in connective tissue metabolism is well understood but the relatively slow turnover of such molecules and the fact that the levels of available sulphate are well able to satisfy the needs of the body may lead to under-valuation. In comparison with phosphorylation, sulphation is a very poor research relation, again in terms of rates of turnover and concentrations, and similar considerations apply to the sulphoconjugation of endogenous and exogenous small molecule sulphate acceptors. However, judgement of importance on the basis of concentration is often unwise.

A number of questions arise in attempting to understand how sulphate availability relates to the metabolism of xenobiotics and how sulphate depletion might affect the response to xenobiotic challenge.

(a) What is the size of the inorganic sulphate pool and how is it controlled?
(b) Is plasma inorganic sulphate freely available to the tissues?
(c) To what extent does dietary inorganic sulphate and organic sulphur contribute to the sulphate pool?
(d) Do all cells have the enzyme machinery to produce inorganic sulphate from organic sulphur and is it the same in all cells?
(e) How does depletion of the sulphate pool caused by xenobiotic conjugation affect the normal sulphoconjugate complement of the body and what are the consequences of perturbation of that complement?

Many early classical studies (reviewed by Dodgson and Rose, ref. 19) delineated mammalian dietary dependence on sulphur-containing compounds. A consolidating interest in sulphur balance and the essential S-containing amino acids has been inevitable owing to the inability of animal cells to reduce sulphate and the pervading role of the SH group in biochemical reactions and structures. Important agricultural implications also inevitably focused attention on dietary inorganic sulphate and its sparing effects on growth-limiting sulphur-containing amino acids. All of these studies have contributed to a

vast store of accumulated information on sulphur balance and nutrition.

More recent work /21,22/ has revived interest in and provided insight into the nature and size of the inorganic sulphate pool. Thus, it is known that serum levels of inorganic sulphate are species dependent (for example, man, 0.3 mM and rat approximately 0.8 mM) that plasma and tissue inorganic sulphate are in rapid equilibrium and that sulphate transport "across some membranes at least" involves a carrier protein embedded in the cell membrane. It has also become clear that contrary to long held views, inorganic sulphate is readily absorbed from the gastrointestinal tract. Similar incorrect folk lore about the non-absorption of sulphoconjugates has also been corrected by experimentation. Other pertinent studies have revealed that the renal threshold for inorganic sulphate is very low and that there are secretory mechanisms for sulphoconjugates in the kidney /23/.

A substantially coherent picture of sulphate availability has emerged and many of the questions posed can now be answered with certainty. However, although the whole-body biochemistry of sulphur and sulphate is virtually established, many problems still remain particularly in assessing the nutritional values of different forms of sulphur in maintaining sulphur balance and the availability of sulphate in different cell types. Further information is vital to explaining the ability of different cell types to produce inorganic sulphate from organic sulphur compounds and on their ability to utilise intermediates in the organic/inorganic sulphate pathways. Such information is critical to understanding the capabilities of various cell types in the production of sulphoconjugates and in forecasting their vulnerability to xenobiotics that are detoxified by sulphation. Much attention has been given to species variation in sulphation ability but many of the unsolved problems of xenobiotic metabolism stem from inadequate information on the availability of sulphate in different cell types and on the organ and intracellular distribution of the xenobiotics themselves.

Other important information relates to cell concentrations of PAPS. PAPS is metabolically labile and tissue concentrations are low (in rat liver 30 n mol/g tissue) but it is also known that rapid synthesis can occur in liver at least (0.1 n mol per min/g) /21/. Recent studies /24/ in cultured cells have significantly added to

knowledge on sulphate generation in different cell types. These ingenious studies, based on the formation of proteoglycans, the major use of sulphate by most cells, have shown that there is a differential capacity of cells to utilise cysteine, cysteinesulphinic acid and sulphite as sulphate sources.

The observation that the formation of sulphoconjugates can be rate-limited by the endogenous level of the sulphate ion was made more than 30 years ago and Dodgson /25/ pointed out in 1977 that in spite of various observations, the general picture of sulphur status in relation to sulphoconjugation is "still inadequately defined and some new experimental approaches are badly needed". Such new approaches have been forthcoming and applied to both endogenous and exogenous sulphate acceptors /26,27,28/ and very significant progress has been made. It is now quite clear that sulphate availability may substantially influence xenobiotic toxicity and the recent studies /29/ in isolated perfused rat and guinea pig livers further illustrate that a xenobiotic load may limit the sulpho-conjugation of endogenous molecules. The full significance of these collective studies is yet to be assessed in terms of whole-body sulphate status but the indications are that this will be a profitable research area.

The roles of sulphohydrolases in mammals reflect the eukaryotic multi-organ nature of the mammalian system and many of their substrates are well characterised. On the other hand it is currently quite difficult to ascribe with any confidence, specific physiological roles to sulphatases produced by microorganisms. Microbes generally do not enter the prolonged steady-state of adulthood typically enjoyed by mammals, but their metabolism is rather geared to a continual round of cell growth and division. In such circumstances it is not unreasonable to suppose (and there is some evidence for this) that a major role of microbial sulphatases is in the acquisition by the cell of nutrients (carbon and/or sulphur) from sulphate esters in the environment.

V. NATURAL SULPHATE ESTERS AND MICROBIAL SULPHATASE SPECIFICITY

The structural diversity of naturally-occurring sulphate esters is extreme and so, necessarily, is the range of specificity encountered

among microbial sulphatases. The quickening of interest in these enzymes can be traced to two main factors: first, the realisation that they could serve as valuable tools if carefully used /see 30, 31/ in the analysis of a variety of natural and synthetic sulphate esters /13/ and second a growing awareness of their importance in returning ester sulphate to the global sulphur cycle /32/.

It is not generally appreciated that sulphate esters are widely distributed in Nature, and in a great variety of structures. Some of the structures already known /13/ include:

(i) arylsulphates;
(ii) alkyl sulphates;
(iii) monomeric and polymeric carbohydrates in which sulphate moieties may be attached at one or more positions in a given monosaccharide;
(iv) a heterogeneous group of compounds in which the carbon in the $C\text{-}O\text{-}SO_3^-$ ester linkage is replaced by phosphorus or nitrogen.

Within each group there is a wide range of chemical diversity and to this list, must be added the almost inumerable and largely unidentified sulphate conjugates excreted by higher organisms /33/.

Continued rotation of the global sulphur cycle, and the notion of microbial infallibility, demand that microorganisms must exist to utilise these compounds as sources of carbon or sulphur or both. In broad terms this has been shown to be the case, and microorganisms have been isolated that are able to degrade representatives from all four classes. Some of these isolates are of enteric origin whereas others are found free-living in habitats such as soil and river-water. In almost all cases studied, sulphate ester degradation involves the action of sulphatase enzymes, usually (though by no means always) as the initiating step in the metabolic pathway. However although the demonstrated range of specificity of microbial sulphatases is wide, detailed specificities hardly begin to match the structural diversity of the esters encountered in nature probably simply due to the fact that the study of the enzymes has been outstripped by the identification of possible substrates.

Some bacterial alkylsulphatases remove SO_4^{2-} from surfactant molecules like dodecyl sulphate (SDS). The point was made earlier that conjugation with sulphate in mammals profoundly alters the properties of the acceptor molecule. Removal of sulphate is of course equally dramatic, in the case of SDS converting a powerful

surfactant capable of disrupting protein and membrane structure, into a relatively innocuous long-chain alcohol. Thus biosynthesis of alkylsulphatases and their location in the periplasmic space of Gram-negative bacteria afford the cells with a very effective detoxication mechanism against potential damage from SDS.

These few assignments of roles, tentative and even speculative as they are, together with the lack of correlation between known structures of naturally-occurring sulphate esters and microbial sulphatase specificity, reflect how little we understand of the microbial biochemistry and physiology of sulphate esters and sulphatase enzymes. Moreover, the possibilities of interplay between microbial and mammalian sulphate metabolism, for example in the gastrointestinal tract are in their infancy.

The multiplicity of roles played in Nature by sulphoconjugates and the enzymes responsible for their conjugation and deconjugation, points to a much wider significance for sulphoconjugation than the detoxication of xenobiotics. Although the significance of sulphate conjugation in inactivating toxins and rendering molecules suitable for excretion, should not be undervalued, it may be important to recognise that sulphoconjugation is but a subsidiary role for the sulphate moiety. Perhaps a suitable analogy is the use of amino acids in conjugation. Like sulphate, these have other major commitments in biological systems.

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