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Biochemistry of the SH Group

The Occurrence, Chemical Properties, Metabolism and Biological Function of Thiols and Disulphides

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Preface

Over a decade ago I was invited by Academic Press to write an account of the biochemistry of glutathione. The project however did not materialize because it became clear that the chief biochemical interest in the reduced and oxidized forms of glutathione was not in the peptides themselves but rather in their relationships with other cellular thiols and disulphides. A worthwhile work would therefore have to consider the latter as a group rather than to concentrate on one particular member.

The present book attempts to achieve this broader concept. My aim has been to give a concise, up to date and integrated account of biochemically relevant types, properties and interactions of thiols and disulphides. The justification for producing it at this time is that, although there is a considerable and growing interest in this field, such knowledge has not previously been collected together in one volume.

The work is intended to be intelligible to general readers with a university first year background in biochemistry and organic chemistry. It should also interest research workers because it has been compiled chiefly from the original papers and most of the 2000 odd references cited are less than 10 years old.

I am indebted to several people who have been involved in the appearance of this book, in particular to the late Dr C.P. Stewart who introduced me to the subject, the late Mr A.L. Bacharach who suggested the commission and Professor R.B. Fisher who encouraged its development. Thanks are especially due to Dr E. Beutler and to Dr P.L. Wermer of the City of Hope Medical Center, Duarte, California for their generous support of the original idea.

My colleagues, Dr D.K. Apps, Dr I.B.R. Bowman, Mr J. Doyle, Mr A. Kamminga, Dr I.A. Nimmo, Dr W.N.M. Ramsay and Dr A.P. Ryle have kindly read particular draft chapters and made useful comments and criticisms. They are of course in no way responsible for the deficiencies which remain. Miss Joyce Bisset, Miss Elizabeth Reid and Miss Linda Sidonio skilfully converted my illegible manuscripts into an acceptable typescript. I am also indebted to Mrs. Jane Duncan of Academic Press who prepared the work for the printer. My wife, Josie, made generous allowances for the 'author syndrome', thereby making the experience of writing this book, at least in retrospect, bearable.

June 1972. P.C. Jocelyn

Introduction

Living organisms require sulphur. Some of them (e.g. the sulphur bacteria) use it as the free element, some (e.g. the plants) as sulphate or sulphide and some (e.g. the higher animals) as the cystine and methionine residues of proteins.

Irrespective of which of these forms is ingested, sulphur nevertheless appears in cells in only three principal chemical fractions which are to some extent interconvertible.

One of these is the sulphide fraction, made up of $-S-CH_3$ groups from methionine residues of cellular proteins. Though chemically not very active this *alkali stable* fraction is by no means inert (p.66).

Sulphur also appears as sulphate bound chiefly as ester or amide sulphate in various polysaccharides and steroids. The relationship between this sulphate and the other forms of sulphur is the subject of a recent book.[1*]

The third fraction of sulphur is that present as cellular SH (sulphydryl) and SS (disulphide) groups. This fraction has been the one most amenable to investigation thanks chiefly to a multitude of methods available for detecting and assaying these groups.

In consequence, there is a welter of factual knowledge about the substances (thiols and disulphides) possessing these groups, much of it obtained over the past decade.

The aim of this book is to present and as far as possible to integrate this knowledge.

HISTORICAL OUTLINE

Interest in the subject originates from 1810 when Wollaston[2] isolated the disulphide-containing amino acid cystine from a urinary calculus (p.4). Another 74 years elapsed before the first thiol, cysteine was obtained from it by reduction,[3] but shortly afterwards the presence of other sulphur-containing substances in organisms was recognized by the preparation of 'philothione' (now known to have been an impure form of glutathione (GSH).[4] Soon afterwards Mörner[5] discovered that cystine was also a product resulting from the hydrolysis of proteins. It was much later, when precautions were taken to prevent autoxidation during proteolysis and when methods had been developed for assaying protein-SH groups, before it was realized that this cystine can arise from the presence in proteins of cysteine as well as cystine residues.

During this early period, the nitroprusside reaction (p.150) had been developed for detecting SH groups. Its application to tissues

gave the first indication[6] that thiols occur in tissue extracts generally, both in the protein and in the non-protein fractions.[7]

The thiol, ergothioneine was isolated from rye in 1909 (p.7) but it has only a weak nitroprusside reaction.

The source of the non-protein SH groups detected by this reaction was eventually found by Hopkins whose discovery and isolation of GSH in 1921–8 (p.11) put the study of SH groups on a firm chemical footing, thus stimulating a great and sustained biochemical interest.

A puzzling dietary problem was posed after the discovery of methionine in 1922[8] when it was realized that methionine residues in proteins are the source of much of the sulphur of SH and SS groups. This was only resolved by 1942 when it was shown by du Vigneaud how methionine can serve as a methylating agent and how the resulting homocysteine can be converted to cysteine (p.169).

The search for more thiols culminated in the discovery of coenzyme A by Lipmann in 1945 (p.9). Then in 1951, Reed isolated the cyclic disulphide, lipoic acid (p.6). Both these substances have coenzyme roles which are lost when their SH groups – in the case of lipoic acid, after conversion to the reduced form dihydrolipoic acid – are oxidized or alkylated.

Meanwhile enzymes were being crystallized and found to possess SH groups necessary for their catalysis. The first two[9*] were urease and papain but these were soon followed by an ever increasing number of others (pp.15–34).

THE FUNCTION OF THIOLS

The idea that thiols as a group might have some common biological function springs from the discovery, first reported by Rapkine in 1931,[10] that SH group concentrations change during cell division. Rapkine used sea urchin eggs but the finding appears to be a general one (p.230) and indicates the existence of an SH/SS cycle during mitosis.

Such changes in the gross concentration of SH groups naturally focus attention on the most abundant of the thiols, namely GSH.

Present in most tissues, a variety of enzymes (p.176) governs the *in situ* rate of biosynthesis and degradation of GSH and of its reduction-oxidation equilibrium with the disulphide form, GSSG. GSH does have a coenzyme role (p.206) but the reactions it catalyses may be merely metabolic curiosities and it cannot be said to be biologically active like the other thiols mentioned. Its function, assuming it is not superfluous in cells, has been and still is a source of puzzlement.

GSH has a well known 'euphoristic' effect (a term used by Racker[11*]) on SH-containing enzymes *in vitro* due to its acting either as a reducing agent or a metal chelating agent.

In a celebrated review in 1951, Barron,[12*] extrapolating from these properties and using the data of Rapkine, put forward the idea that stimulation of SH-enzyme activities by GSH also occurs intracellularly and is "one of the regulatory mechanisms of cellular respiration".

It was implied that tissue SH-enzymes are partly in an inactivated state due to the presence of natural inhibitors. Oxygen and various disulphides could fulfil this latter role for both are known inhibitors for some such enzymes *in vitro* (p.36).

The theory inspired such work as, on the one hand, the effect of hormones (p.312), vitamins (p.306) and various disease states[13*] on SH-group concentrations and on the other, the relationship between the SH-groups in SH-containing enzymes and their catalysis (p.203).

However it is not now tenable partly because of the results of such work and partly because of advances in other fields. Some of the objections are as follows.

(1) More specific methods of analysis have failed to confirm some of the earlier findings of elevated SH group concentrations. Those found by Rapkine have been confirmed but are not, as was originally thought, due to GSH (p.230).

(2) Many enzyme SH groups are superfluous to catalytic activity and their inhibition by substances reacting with SH groups is due to the substituent introduced on to the sulphur – and so depends on the reagent used – rather than to the loss of the SH group itself.

(3) The question of how, if it has such a controlling influence, the concentration of SH groups is actually varied in cells remains unanswered. No systematic changes in the activity of enzymes metabolizing GSH have been found. In any case the idea that such concentration changes could have a controlling influence throughout the cell is probably not compatible with present knowledge about cell compartmentation.

Despite such objections, the concentration of SH groups and their metabolism in different parts of the cell remains of biological interest and is still considered by some to be of fundamental importance. For instance, it was possible to maintain in 1967 that "oversynthesis of specific SH-producing enzymes may be an essential feature of carcinogenesis."[14*]

However, it is now more likely that such changes, though often unexplained, are the consequence not the cause of more deep-seated ones.

COMMON PROPERTIES OF THIOLS AND DISULPHIDES

The chief reason for considering thiols and disulphides together at the present time is that they have in common, and owe their many different biochemical roles to, the remarkable chemical properties of SH and SS groups.

SH groups, unless masked as in some proteins, are chemically the most active groups found in cells and as such they react as a class specifically and often quantitatively with various thiol-combining agents. The latter include a wide range of metal ions with which they form stable complexes.

Thiols dissociate and their reactivity is in fact due to RS′ ions which are over 500 times more nucleophilic than the corresponding oxygen analogue, RO′[15]*. RS′ may also lose an electron to give unstable free radicals, RS· and these may propagate or terminate free radical chain reactions.

Sulphur atoms link together as linear chains in the polysulphides but they are reluctant to form double bonds with themselves or with the atoms of other elements. One important consequence of this is that the thiocarbonyl group ($\geq C = S$) is not stable and accounts for the special properties of thioesters.

Perhaps the biologically most interesting property of SH groups is that they can oxidize.

Sulphur has valencies ranging from 2 to 6 so that several types of oxidation product are possible. However the products most easily formed are the disulphides.

Disulphides are much less active than SH groups and function as stable elements of structure in proteins. However various reagents can cleave them (Chapter 5) and so convert them back to SH groups or their derivatives.

The reduction of a disulphide can be performed by another thiol so that the SH groups of one thiol can in principle react with the SS group of a different disulphide (p.121).

This thiol-disulphide exchange reaction is in fact a most important unifying concept which dominates the biochemistry of thiols and disulphides. Several examples of its occurrence in cells are described throughout this book (pp.166, 192, 231, 234, 247, 280, 307, 341).

THE SCOPE OF THIS BOOK

Before considering the chemistry and biochemistry of thiols and disulphides, based on these common properties, in detail, it is of interest to know what substances actually possess SH and SS groups and what are their salient individual properties. This is the subject of Chapter 1 which describes or lists the known non-protein and protein

thiols and disulphides, the latter ranging from enzymes to insoluble proteins such as hair. The common physical properties and chemical reactions of SH groups (Chapters 2, 3 and 4) and SS groups (Chapters 2 and 5) are then considered, followed by an account of the methods used for their analysis (Chapter 6).

Thiols and disulphides, in common with other cell constituents, are constantly being degraded and resynthesized. Cysteine is synthesized *de novo* or from methionine and degraded to give sulphate. It is also the source of the SH group for the biosynthesis of most of the other thiols.

These metabolic inter-relationships are presented in Chapter 7.

The general functions of thiols and disulphides in cells (Chapter 8) can be considered under three heads; their role as redox reagents which enables them to function in various electron transport systems, their role as catalysts in enzymes and coenzymes and their role as detoxicating agents.

From a biological standpoint, thiols and disulphides have particular functions in different subcellular particles (Chapter 9) and in different tissues (Chapter 11) including blood (Chapter 10).

In these locations thiols and disulphides can interact with other cell constituents especially with coenzymes and hormones.

The extent to which they do so is considered in Chapter 14.

These aspects of thiols and disulphides refer chiefly to animals but they are also required by and have somewhat different functions in plants (Chapter 12) and micro-organisms (Chapter 13) where particular emphasis is placed on the role of cysteine in the biosynthesis of the sulphur-containing coenzymes and antibiotics.

Finally, the biological effect of giving various synthetic thiols and disulphides to organisms is described. A great deal of research has been done on radioprotection by thiols, shown most of all by cysteamine and its corresponding disulphide cystamine (Chapter 15). Different and characteristic effects are also shown by the disulphide drug dithiouram (antabuse) and by the thiols, 2,3-dimercapto-propanol and penicillamine (Chapter 16).

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NON-STANDARD ABBREVIATIONS

CMB. *p*-chloromercuribenzoic acid

DTNB. 5,5′-dithiobis(2-nitrobenzoic acid)

E.C. Enzyme Commission. The recommendation of the Report of the Commission on the numbering of enzymes have been followed. (The report is available in 'Comprehensive Biochemistry', (1965). Vol. 13. Ed. by Florkin, M. and Stotz, E.H. Elsevier.)

NEM. *N*-ethylmaleimide

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