Review Letter

HOW MANY DISTINCT ENZYMES ARE RESPONSIBLE FOR THE SEVERAL CELLULAR PROCESSES INVOLVING THIOL:PROTEIN-DISULPHIDE INTERCHANGE?

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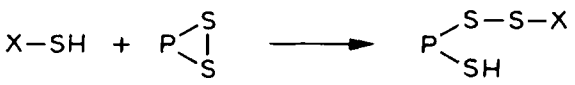
1. Introduction

The reaction:



is an oxidoreduction involving a thiol and a protein disulphide, or more simply a thiol:protein-disulphide interchange. Reactions of this kind are components of several rather diverse cellular processes most of which are poorly characterised. In particular, few enzymes catalysing thiol:protein-disulphide interchange have been described in detail and their physiological functions are still subjects for debate. This review aims:

(i) To set out the different cellular processes involving thiol:protein-disulphide interchange and to argue that these suggest the existence of several enzyme species;
(ii) To review what is known about enzymes catalysing such reactions;
(iii) To consider the problems in assigning physiological roles to such enzymes;
(iv) To suggest how ambiguities about physiological function may be minimized and how the various enzymes catalysing thiol:protein-disulphide interchange in a specific source may be enumerated.

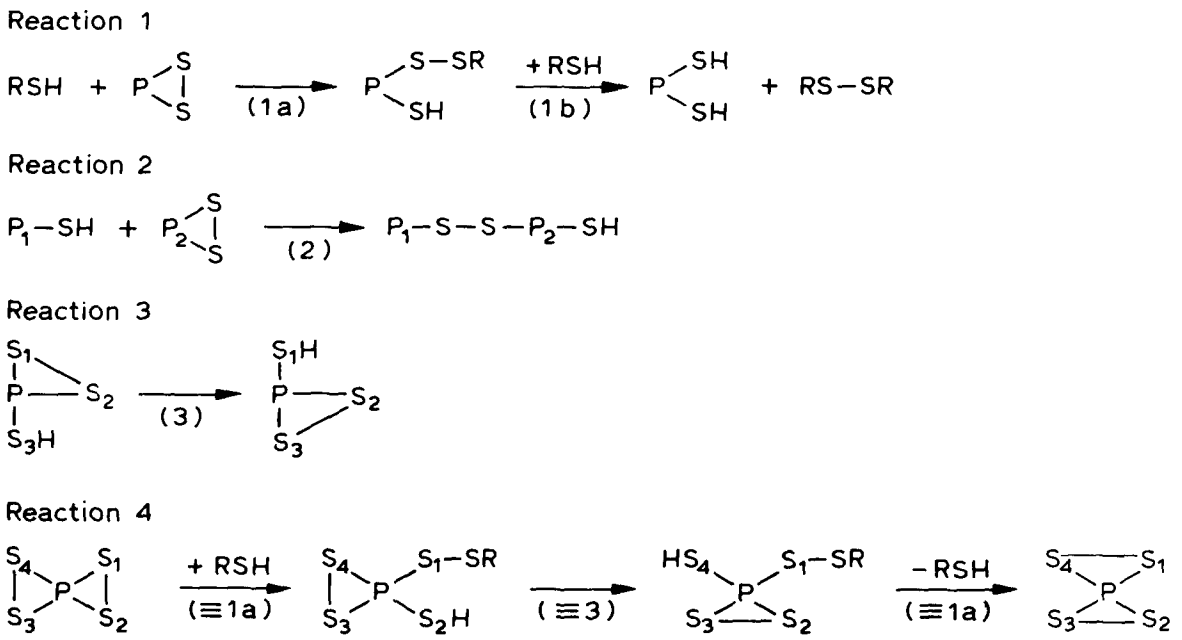
2. Diversity of processes involving thiol:protein-disulphide interchange

Depending on the nature of the thiol compound, thiol:protein-disulphide interchanges can have rather different overall consequences. With a low molecular weight thiol, the product is a mixed disulphide and protein thiol (reaction 1a) and further reaction with a low molecular weight thiol will lead to complete reduction of the protein disulphide (reaction 1b). If the thiol is a component of a distinct polypeptide chain, the interchange reaction leads to the formation of an interchain disulphide bond (reaction 2), whereas if the thiol is located in the same polypeptide chain as the disulphide, the consequence is an intramolecular rearrangement of disulphide and thiol groups (reaction 3). A combination of reactions (1a) and (3) can lead to the complete isomerization of disulphide bonds in a fully oxidised protein (reaction 4). Examples of all these processes have been observed in vitro, and they have been proposed to be involved in numerous cellular events.

2.1. *Reduction of protein disulphides and mixed disulphides*

Studies on the metabolism of polypeptide hormones, especially insulin, have indicated that reduction of their disulphide bonds plays an important part in their degradation [1–7]. Studies on insulin degradation using whole cells [7], homogenates [1–3,5,6] and sub-cellular fractions [4,8] show that the reduced A chain and various ill-characterised oligomers of the A and B chains are major products. Considerable evidence has been advanced showing that a two-step pathway of degradation – reduction followed by proteolysis – occurs at both high and at physiological insulin concentrations [5–7]. Although this is not universally accepted as

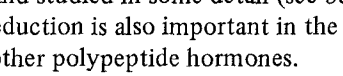
Scheme 1



the obligatory pathway of insulin degradation (see, e.g., [9]) it is certainly the case that many tissues have the capacity to reduce insulin. It has always been assumed that glutathione, the predominant low molecular weight intracellular thiol compound, is the reductant in this process. A glutathione:insulin oxidoreductase, commonly known as glutathione–insulin transhydrogenase (GIT) has been identified in several sources and studied in some detail (see below).

Disulphide reduction is also important in the metabolism of other polypeptide hormones. Glutathione:oxytocin oxidoreductase activity has been detected in mouse mammary tissue [10] and human placental tissue [11], and the human placental activity has been shown to have a considerable specificity for oxytocin and vasopressin and quite a low activity with insulin. Neither of these systems has been characterised in any detail.

The assumption that glutathione or a related low molecular weight thiol is the major reductant of protein disulphide bonds may not be wholly justified. The thioredoxin system, characterised by Holmgren and others (reviewed [12]) may also play a part. Thioredoxin, a protein of mol. wt 12 000 was first discovered as a component of the system in *Escherichia coli* responsible for the reduction of ribonucleotides to deoxyribonucleotides [13]. The reduced form of thioredoxin – thioredoxin–$(SH)_2$ – is the immediate reductant in this process; the dithiol is a strong reductant (redox potential –0.26 V at pH 7, 25°C) and is converted to a product with an intramolecular



disulphide bond [14].

Thioredoxin is widely distributed in bacterial, lower eukaryote and mammalian sources [15].

The reduction of thioredoxin-S_2 is brought about by an NADPH-dependent process involving the flavoenzyme, thioredoxin reductase [16]. The combination of NADPH, thioredoxin reductase and thioredoxin-S_2 in vitro, is capable of reducing disulphide bonds in low molecular weight compounds, such as cystine and in polypeptides such as insulin and human choriogonadotropin [16,17]. The system may be of considerable importance in reduction of disulphides in vivo but its role is not yet established.

It has recently been shown that the light-dependent regulation of the activity of key enzymes of CO_2 assimilation in chloroplasts involves thioredoxin. Thioredoxin is reduced photochemically via ferredoxin and a ferredoxin–thioredoxin reductase, and the reduced thioredoxin reduces (and activates) fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase and possibly other enzymes [18]. In the dark, these enzymes are reoxidised (and inactivated), possibly by oxidised glutathione. In this scheme, both the activation and inactivation are thiol: protein-disulphide oxidoreductions [18]. This finding emphasizes the diversity of thioredoxin function. Mammalian liver thioredoxin is active in the reduction of disulphides in mammalian proteins, but also replaces bacterial thioredoxin in the reduction of ribonucleotides to deoxyribonucleotides and replaces chloroplast thioredoxin in the photoregulatory process outlined above [19].

Mixed disulphides between proteins and glutathione or cysteine are present in quite high intracellular concentrations. In several rat tissues, the concentration of protein-SSG is 25–50% of the concentration of total acid-soluble thiol groups [20]. In rat liver, the concentration of protein-SSG at times exceeds that of GSH (see below). Thus a considerable proportion of intracellular glutathione is in the form protein-SSG and this must play a part in the redox buffering effect of glutathione [21]. For example, exposure of rats to ozone leads to a fall in free glutathione in the lung without any increase in GSSG; the fall in GSH is correlated with an increase in the level of protein-SSG [22].

The level of protein-SSG in rat liver shows a clear-cut diurnal variation, inversely correlated with a variation in the level of GSH [23]. The concentration of protein-SSG is maximal (17 nmol/mg protein) at 6 p.m. and minimal (5 nmol/mg) at 6 a.m. whereas that of GSH is minimal at 6 p.m. (13 nmol/mg) and maximal (25 nmol/mg) at 6 a.m. These changes may result from the diurnal variation in level of cAMP [24]. In rats fed ad libitum, the ratio of protein-SSG to GSH rises during the period when hepatic cAMP levels are high and drops when hepatic cAMP levels are low; furthermore, the ratio of protein-SSG to GSH in rat liver is increased by intraperitoneal injection of dibutyryl-cAMP. The fact that the activities of several enzymes of carbohydrate metabolism (notably fructose-1,6-bisphosphatase and phosphofructokinase) are altered by formation of mixed disulphides (see [24,25]) suggests that this formation of mixed disulphides in response to diurnal variation in cAMP levels may have regulatory significance.

So the formation of protein-SSG mixed disulphides may contribute to the redox buffering of the cell, the prevention of oxidant toxicity, and the modulation of the specific activity of certain proteins. In any case, the existence of such mixed disulphides implies the existence of a system for reducing them to generate the thiol group on the protein [26]. In crude systems these mixed disulphides are reducible by NADPH, but this is probably an indirect effect. There is good evidence that protein mixed disulphides are directly reduced by GSH in a thiol:disulphide oxidoreduction [27]; the requirement for NADPH in crude systems then arises from the necessity to regenerate GSH via glutathione reductase. The GSH: mixed disulphide oxidoreductase has been given the trivial name 'thioltransferase' [28] and characterised to some extent (see below).

2.2. *Formation of interpolypeptide disulphide bond*

Disulphide bonds between polypeptide chains are present in very many classes of extracellular proteins including digestive enzymes, immunoglobulins, mucus glycoproteins and procollagens. Evidence is accumulating that certain proteins in plasma membranes may also exist in extensive disulphide crosslinked networks [29,30]. The mechanism of the formation of these bonds in vivo is obscure.

The formation of interchain disulphide bonds has been most intensely studied in the case of the IgG immunoglobulins which consist of two heavy (*H*) chains and two light (*L*) chains linked by several disulphide bonds to give an *L–H–H–L* structure. The course of assembly of this structure in vitro has been compared to that observed in vivo. However, much of the work on this assembly in vitro has used the non-physiological oxidant O_2 [31,32] and even in cases where GSSG was employed as oxidant [33] the starting material was fully reduced but non-covalently associated *H* and *L* chains. So from these model studies it is difficult to draw conclusions as to the chemical events involved in in vivo assembly. However, the bulk of the evidence favours thiol:

protein-disulphide interchange as the crucial reaction. Thus, in the GSSG-dependent coupling of *H* and *L* chains [33], the pathway appears to be:

$$H\text{-SH} + \text{GSSG} \rightarrow H\text{-SSG} + \text{GSH};$$

$$H\text{-SSG} + L\text{-SH} \rightarrow H\text{-SS-}L + \text{GSH}$$

Similarly, the formation of this 'half-molecule' can occur from reduced *H* chains and *L* chain dimers, a reaction which may occur in vivo since many cells synthesize *L* chains in excess over *H* chain and the excess *L* chain appears as the disulphide-linked *L* -SS- *L* dimer [34]. The reaction is probably a simple thiol:protein-disulphide interchange of type (2):

$$H\text{-SH} + L\text{-SS-}L \rightarrow H\text{-SS-}L + L\text{-SH}$$

In the case of polymeric immunoglobulins IgA and IgM, although the process of assembly is more complex, comparison of in vivo and in vitro data is easier because of the special role of the *J* chain. IgM consists of 5 H_2L_2 units linked by disulphide bonds between heavy chains. In the non-assembled form, IgMs, which accumulates intracellularly these residues are not found as free cysteine, but form an intramolecular disulphide bond [35] (see fig.1). Experiments on the assembly of IgM from reduced chains or from IgMs in vitro have shown an essential requirement for the *J* chain [36], and structural work on IgM has established that this chain forms a clasp in the cyclic pentameric structure [37]. The mechanism of assembly can therefore be postulated to be a thiol: disulphide interchange reaction of type (2) (see scheme 1). Further evidence for this is the finding that the assembly of IgM in vitro is dependent on the presence either of microsomal membranes or of protein-disulphide isomerase [36,38] an enzyme isolated from microsomal membranes which catalyses thiol:protein-disulphide interchange (see below).

Little is known about the process of interchain disulphide bond formation in other secreted proteins. In the case of procollagen, the evidence suggests that interchain disulphide bond formation occurs in the cisternae of the endoplasmic reticulum [39]. In this connection, it is interesting that protein-disulphide isomerase has been detected in microsomal preparations from most sources, including collagen-synthesizing tissues [40]. However, in the case of mucins the evidence is that disulphide formation is a late intracellular event, just preceding secretion [41].

2.3. *Intramolecular thiol:disulphide interchange*

Few proteins contain both disulphide bonds and free thiol groups [42]. In those cases where both coexist, there is the possibility of intramolecular thiol:disulphide interchange. Bovine serum albumin is a classic case, and there is now good evidence that the heterogeneity of this protein in isoelectric focussing arises from the existence of various disulphide-interchanged isomers [43]. There is also the interesting possibility that intramolecular thiol:disulphide interchange is of regulatory significance. Thiol proteases often contain disulphide bonds and it has been proposed that in papain, intramolecular thiol: disulphide interchange involving the active site thiol (Cys-25) and one of the enzyme's 3 disulphide bonds could participate in a zymogen activation system [44]. Brocklehurst and Kierstan [44] propose that propapain, a catalytically-inactive protein found in

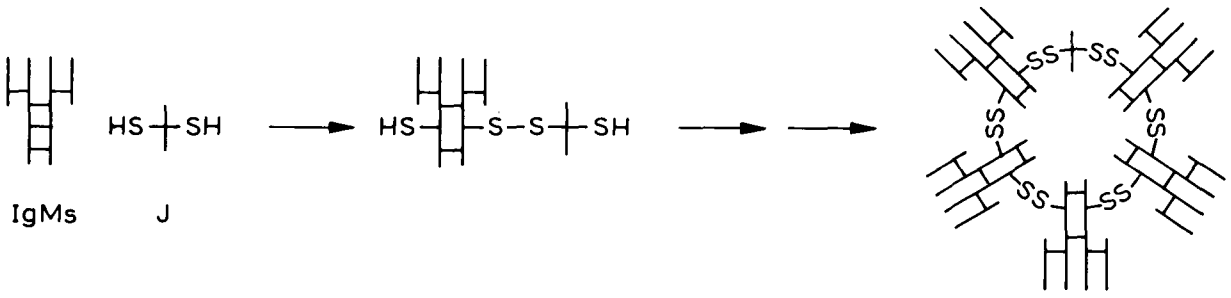


Fig.1

papaya latex is a storage form of papain and differs only in that the Cys-25 residue is involved in a disulphide bond, probably with either Cys-22 or Cys-63.

Tyrosine aminotransferase from rat liver exists in 3 separable forms, which may be relevant to the in vivo regulation of the enzyme. The forms are interconvertible in vitro, and the nature of the differences between them, and of factors which catalyse their interconversion, have been studied intensively [45–47]. At present the evidence suggests that the forms differ in free thiol content and that the cellular systems catalysing their interconversion are catalysts of thiol:disulphide interchange [45].

Intramolecular thiol:disulphide interchange is also important in the oxidation of reduced proteins in vitro and, presumably, in the formation of intramolecularly disulphide bonded proteins in vivo. The best-characterised system is the re-oxidation of reduced pancreatic trypsin inhibitor by low molecular weight disulphides or disulphide/thiol buffers. A pathway for formation of the 3 native disulphide bonds – 5–55, 14–38, 30–51 – has been identified. Of the 5 possible species with one disulphide bond only two (Cys 5–Cys 30) and (Cys 30–Cys 51) are formed and these are rapidly interconverted by thiol:disulphide interchange [48]. A similar process occurs at the level of the 'two-disulphide' intermediates [49]. This penetrating study has many implications for the process of protein folding, but in this context its significance is that it shows clearly the importance of intramolecular thiol:protein disulphide interchange in the formation of protein disulphide bonds.

For larger proteins with more disulphide bonds the pathway of disulphide formation is not known in such detail. Bovine serum albumin contains 17 native disulphide bonds and one thiol group; theoretically $>10^{20}$ differently disulphide-bonded isomers can arise from the fully-reduced protein. The rate of reoxidation and folding of serum albumin has been studied in vitro using antibodies as probes of regain of elements of the native conformation [50–52]. The findings show that reoxidation is catalysed by GSH and by a rat liver microsomal preparation showing protein-disulphide isomerase activity (see below). Native molecules are reformed, but the process is slower than for smaller proteins [50]. Different regions of the molecule regain the native conformation at slightly different rates. Individual domains also refold to the native conformation and some evidence suggests that the isolated domains, obtained by partial proteolysis, refold faster than the corresponding regions in the intact protein, presumably because of the lesser probability of forming non-native disulphide bonds between domains [51,52]. The conclusions are broadly confirmed by work on human serum albumin [53] which also shows that in the early stages of refolding much of the albumin is present as oligomers, which slowly convert to monomer as reoxidation proceeds.

2.4. *Protein-disulphide isomerization*

In addition to the intramolecular interchange just considered, disulphide bonds can isomerize even in fully-oxidised proteins, in a process involving mixed disulphide formation (reaction 4). This reaction may be important in the formation of native disulphide bonds both in vitro and in vivo.

The formation of intramolecular disulphide bonds from reduced proteins has been intensively studied in vitro, mainly to throw light on the process of protein folding and the formation of the native conformation. The nature of the oxidant in vivo is not firmly established [54], and in the model studies low molecular weight disulphides [55,56], transition metals in solution [57,58] and metalloproteins [59] have all been used as oxidants. Ribonuclease has been studied in most detail and the time course of oxidation (loss of free –SH groups) has been compared to the time course of conformational changes and the regain of native enzyme activity [56,60]. Although a certain amount of activity is associated with the fully-reduced enzyme [61] the time course of reoxidation is considerably faster than that of reactivation [56,60]. The inactive material after short periods of oxidation consists of a large number of fully oxidised species containing non-native disulphide pairings, which then rearrange to the native state slowly via reaction (4) [56]. This is the process catalysed by protein-disulphide isomerase. Conformational changes accompanying this rearrangement have been detected. [56,62]. The oxidation of reduced lysozyme by 40 μM Cu^{2+} likewise gives rise to inactive oxidised forms which are slowly converted to the native form by thiol:disulphide interchange [58].

3. Enzymes catalysing thiol:protein-disulphide interchange

In the previous section, 3 enzymes are mentioned which catalyse thiol:disulphide interchange. They will now be considered in turn.

3.1. *Thiol:disulphide oxidoreductase*

The first was discovered in studies on the metabolism of low molecular weight disulphides and mixed disulphides [26]. Most physiological and non-physiological disulphides can be reduced in a reaction involving thiol:disulphide interchange with GSH catalysed by a thiol:disulphide oxidoreductase (termed CySSG-thioltransferase by Eriksson et al. [28]). There appears to be a single non-specific enzyme catalysing this thiol:disulphide interchange involving low molecular weight disulphides, and it is distinct from glutathione reductase and the glutathione *S*-aryl-transferases [28]. The enzyme partially purified from rat liver cytosol is also active towards a protein-SSG mixed disulphide and towards some protein disulphides [27]. A partially purified preparation from yeast has preference for GSH:cystine oxido-reduction but also catalyses reduction of bonds in insulin and serum albumin [63].

Thiol:disulphide oxidoreductases with specificity for protein disulphides are considerably less well understood. Essentially two activities have been used for their analysis namely the reduction of insulin by GSH, to yield GSSG and reduced A and B chains [1–8] a type (1) reaction, and the thiol-dependent regain of enzyme activity from fully oxidised incorrectly disulphide-bonded ribonuclease ('scrambled' ribonuclease), a reaction of type (4) [64–75]. These two activities have been formally named glutathione: protein-disulphide (insulin) oxidoreductase (EC 1.8.4.2) and protein-disulphide isomerase (EC 5.3.4.1), respectively. The former is usually referred to as glutathione insulin transhydrogenase [1].

The problem of enzyme nomenclature is that we characteristically name an enzyme (i.e., a distinct protein species) by the reaction it catalyses, and conversely we are inclined to define an activity by a full enzyme title and therefore to assume we are referring to a distinct protein species. But when we assay an activity in a crude preparation we cannot tell whether one or several species contribute to that activity, and therefore we cannot tell whether the properties we observe are those of a definable 'enzyme'. In discussing the properties of glutathione insulin transhydrogenase and protein-disulphide isomerase it is therefore useful to distinguish between observations on crude systems and on reasonably pure preparations.

3.2. *Protein-disulphide isomerase*

Assayed by the ability to reactivate scrambled ribonuclease, protein-disulphide isomerase has been detected in all animal tissues studied and in plant material [42,64–67]. In liver, the microsomal fraction has the highest specific activity [64,68,69] and the enzyme has been purified from this source [70]. Anfinsen et al. described material purified to homogeneity from beef liver, which has mol. wt 42 000, contained a single free cysteine group and a disulphide bond [71] and catalysed regain of native activity from several reduced or scrambled proteins [68,70,72]. The purified material also catalysed the interchange of disulphide bonds in insulin leading to the formation of complex aggregates [73]. (No information was published on whether this material showed glutathione insulin transhydrogenase activity.) This work all dates from the period 1963–1967 and has not been repeated or extended since then.

More recent studies of protein disulphide isomerase have not used material purified to homogeneity [36,50,69,74–77]. However partially-purified material (see [69]) has been shown to catalyse assembly of IgA and IgM from partially-reduced species [36] and reoxidation of reduced bovine serum albumin [78]. It also shows some glutathione insulin transhydrogenase activity but this is either a trace contaminant or a side reaction catalysed by the isomerase species since although the isomerase activity was purified 140-fold, the transhydrogenase was only purified 10-fold relative to the homogenate [69]. (We have subsequently used ion-exchange and covalent chromatography to obtain preparations of protein disulphide isomerase with even lower relative levels of glutathione insulin transhydrogenase activity [79].)

3.3. *Glutathione insulin transhydrogenase*

Glutathione insulin transhydrogenase has been studied more extensively, and Varandani's group, in particular, has provided much information on the

tissue [80] and subcellular [80,81] distribution of this activity and its response to changes in physiological status [82,83]. Several groups have described purifications of the activity from liver or pancreas [4,84–87] but there is little agreement on molecular properties, and as has been recently pointed out [87] it seems likely that many of these preparations are 'contaminated with other proteins or partially degraded thiol:protein-disulphide oxidoreductases'. All preparations of glutathione insulin transhydrogenase which have been described show protein-disulphide isomerase activity but in no case has a quantitative assessment been made of the relative purification of this and the glutathione insulin transhydrogenase activity.

4. One, two, many?

The coexistence of glutathione insulin transhydrogenase and protein disulphide isomerase activities in (partially) purified preparations has led to the persistent suggestion [85,86,88] that a single enzyme is responsible for both activities. However, co-purification studies and studies on the responses of the two activities to various treatments shows that this cannot be the case, at least for rat liver and beef liver [69,74, 79]. It is more likely that several enzymes catalysing thiol:disulphide interchange with rather different specificities are present in liver tissue, so that an assay of either the transhydrogenase or the isomerase activity in a crude preparation involves contributions from several enzyme species. This seems most plausible in view of the survey above which indicates that thiol:protein-disulphide interchange reactions may be involved in numerous cellular processes; the reductive degradation of polypeptides and proteins, the regulation of enzyme activity and the maintenance of intracellular redox buffering through the reversible formation of mixed disulphides, the assembly of secreted proteins by the formation of interchain disulphide bonds, the regulation of enzyme activity by the isomerisation of disulphide bonds, and the formation of native disulphide bonds within proteins following synthesis. All such reactions involve initial attack by a thiolate ($-S^-$) species, and are slow at physiological pH, although reasonably rapid at pH 8 and above [89]. These reactions are therefore likely to be enzyme-catalysed and consideration of the number of processes mentioned above, suggests that many enzymes may be involved in the catalysis of thiol:protein-disulphide interchange reactions.

One could adopt alternative hypotheses at the outset:

(i) In view of the large number of different proteins which may undergo disulphide formation, isomerization and reduction and the large number of different disulphide bonds they can contain, one might assume that a correspondingly large number of enzymes existed;
(ii) One could assume that this situation necessitated the existence of a single non-specific enzyme;
(iii) One could distinguish between the different types of thiol:protein-disulphide interchange reaction, as in scheme 1 above and assume a different enzyme for each reaction type.

The first assumption implies the existence of an implausibly large number of distinct enzymes. The second is possible but implies little flexibility. The third is again possible, but there would be problems of specificity; an enzyme capable of catalysing certain thiol:disulphide interchange reactions would surely catalyse others, even though it might show a distinct substrate preference.

Such speculation is clearly of limited value. To analyse such a situation requires the application of a range of different assays involving different thiol and disulphide substrates, covering at least the range of reactions in scheme 1. Such an approach has proved useful in analogous situations. For example, a wide range of glutathione *S*-transferase activities have been recognized in rat liver preparations; study at various levels of purification using a wide variety of substrates has shown that all the various activities reside in 5 distinct enzyme species which have distinct, but overlapping, patterns of substrate preference [90]. Similarly, it is now clear that the multiple monooxygenase activities in liver microsomes do not correspond to an immense number of distinct enzymes, nor to a single non-specific enzyme. A modest number of cytochrome *P*-450 species can now be distinguished, which show different substrate preferences [91,92]. This field has been easier to resolve because of the specific induction of certain cytochrome *P*-450 species by particular inducers.

But such an analysis has not yet been carried out for the thiol: protein-disulphide oxidoreductases. The

major assay systems used (reactivation of ribonuclease, and degradation of insulin) are not very flexible and so there have been no quantitative accounts of the substrate preferences of a purified enzyme preparation as compared to its crude source. As a result, of course, it is difficult to be confident of the physiological roles of the various enzyme species which have been purified. The fact that a preparation has been mainly studied in terms of a particular activity does not guarantee that this activity represents its physiological function [88].

For example, a preparation of protein-disulphide isomerase from beef liver [69] was mentioned above. This clearly has specificity for a reaction of type (4) compared to several of type 1, in that relative to a liver homogenate it has 140-fold higher specific activity for reactivation of ribonuclease, but only 10-fold higher specific activity in the glutathione insulin transhydrogenase reaction [69], and in other GSH:disulphide oxidoreductions [79]. But this specific preference for protein disulphide isomerization over other kinds of thiol:disulphide interchange reactions does not prove that the enzyme in this partially purified preparation is responsible for ensuring native disulphide bond formation during liver protein biosynthesis [93]. The use of a small, fully-oxidised, single-domain pancreatic protein substrate to assay 'protein-disulphide isomerase' is convenient, but wider studies perhaps with reduced albumin, or even reduced fibrinogen are still needed to clarify the mechanism of disulphide formation in biosynthesis of liver protein.

Similarly, the purified preparations of 'glutathione insulin transhydrogenase' have not been quantitatively studied in terms of specificity. In no case has sufficient data been presented to allow one to say that a preparation of glutathione insulin transhydrogenase is more purified in glutathione insulin transhydrogenase activity than in any other thiol: disulphide interchange activity [86]. In fact it is possible that these preparations are specifically enriched in protein-disulphide isomerase activity since the published purifications of 'glutathione insulin transhydrogenase' involve solubilisation with deoxycholate [4] or acetone extraction [80] procedures which activate protein-disulphide isomerase but either inactivate glutathione insulin transhydrogenase or activate it only slightly [69, 94].

5. Conclusion

Because of the nature of the reaction they catalyse, enzymes responsible for the numerous cellular processes involving thiol:protein-disulphide interchange are likely to be active in a range of reactions even when purified to homogeneity. Therefore, to establish the physiological role of any one such enzyme it will be essential to characterise it fully and quantitatively in terms of its activity in the reactions types 1–4 and with a range of substrates. This requires the development of a number of assays (e.g., for reactions types 2 and 3) with a spread of substrates. Such a development will allow the enumeration of the enzymes catalysing thiol:protein-disulphide interchange and the elucidation of their physiological roles.

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