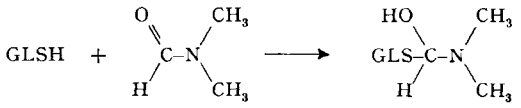
quickly to a jelly) is due to an addition of the SH group across the carbonyl group of the N,N-dimethyl-formamide using dimethyl formamide as an aldehyde for semi thio-acetal formation as a first step as follows:



A similar structure has been proposed by WIELAND et al.[20] for a postulated intermediate in transacetylations with acetylthiophenol[25].

Glutathione sulfoxide VI. GLSO was obtained by dehydrogenation of GLDHSO II with DMSO (dimethyl sulfoxide). 500 mg II were dissolved in 5 ml DMSO. The DMSO was removed by vacuum destillation (1 mm Hg) repeated twice with addition of 5 ml N,N-dimethylformamide. To the somewhat fluid residue 3 ml of a one-to-one mixture of N,N-dimethylformamide and n-butylacetate was added. It crystallized slowly, m.p. 158–161°. The resulting sulfoxide gives no color reaction with nitroprusside. It does not reduce dichlorophenol-indophenol in dry methanol. The sulfoxide is however slightly hygroscopic. In the presence of humidity it reduces dichlorophenol-indophenol slowly. The reduction becomes visible on paper after more than 10 min. The GLSO obtained in this way is soluble in DMSO, N,N-dimethylformamide, water and slightly in alcohol.

Zusammenfassung. Durch Oxydation von Glutathion mit Wasserstoffsuperoxid in Ameisensäure wurde Glutathion-dihydrosulfoxid erhalten. Glutathion-sulfoxid, N-Acetylglutathion und assoziiertes (reversibel polymerisiertes) Glutathion wurden als Vergleichssubstanzen erstmalig hergestellt.

G. E. UTZINGER[27], L. A. STRAIT, and L. D. TUCK

Department of Pharmaceutical Chemistry and Spectrographic Laboratory, The University of California, School of Pharmacy, San Francisco (California, U.S.A.),
November 26 1962.

[25] The significance of covalent intermolecular association lies in the creation of a weak C-S bond which might compete with hydrogen bonding[26] in the connection of proteins to strands. It is better understandable, that the associated bond is opened by the frequently used carbonyl-containing reagents like urea[9] and CO than that a thiol could be hindered from a reaction by a hydrogen bond. It may, further turn out to be part of the *inter*molecular sulfur bonds now attributed to the disulfide bonds.

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Biological and Pharmacological Significance of the Expanded S-Outer Shell in Electron Transfer Reactions

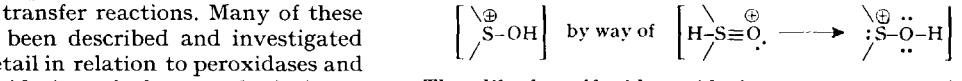
A peptide dihydrosulfoxide[1] having in the same molecule oxidizing and reducing properties such as those present in hydrogen peroxide is given added significance in that it provides, in retrospect, a possible mechanism such as has been sought in the photosynthesis and in anabolic and metabolic electron transfer reactions. Many of these reactions have already been described and investigated in various degrees of detail in relation to peroxidases and catalases[2–4] and the oxidation of photosynthetic intermediates[5].

Further the presence of a carbanion in the α-position of a dihydrosulfoxide provides a 'handle' for the enzymic addition of that carbanion to polar groups like carbonyl groups of substrates or intermediates. Electron transfer within this addition compound appears as an intramolecular rearrangement which may then be followed by dissociation or transfer of the intermediate substrate in a changed oxidation state.

Anaerobic metabolism is, according to WARBURG[6], the effective means by which cancer cells supplement their energy needs. WARBURG[7] has shown that anaerobic metabolism may be inhibited equivalently either by irradiation or by action of H_2O_2. An oxidized cystine has been tested on mice as a cancer growth-retarding agent with partially successful results[8]. It is of further interest in this connection that TOENNIES[9] performed the oxidative conversion of casein in hydrogen peroxide-formic acid mixtures into a protein free of methionine and modified in cystine content.

There are many glutathione oxidizing enzymes reported[10] in the literature which, however, invariably refer to the disulfide oxidation state, which seems to be the predominant form obtained by oxidation in aqueous solution. The usefulness of such enzymes in accomplishing conversion to the dihydrosulfoxide in the semisolid state should be investigated.

A satisfactory simple oxidizing agent has been long sought in photosynthesis, for the oxidation of, e.g., a glycolyl fragment to glycolate. The properties of a dihydrosulfoxide can be shown to satisfy a need to obtain by fission from an energy rich intermediate an oxidizing and a reducing agent, because it can react as hydride ion and



The dihydrosulfoxide oxidation state may satisfy a model for an effect occurring at the sulfur function in the cytochrome of chloroplasts if one postulates that the *in vitro* effect of H_2O_2 in formic acid may mimic photolysis of H_2O. The photo excitation would serve the equivalent of promoting an electron from a *p*-orbital in the sulfur

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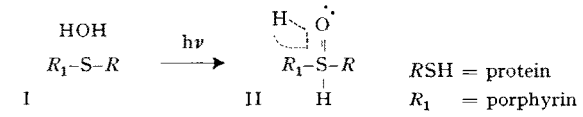
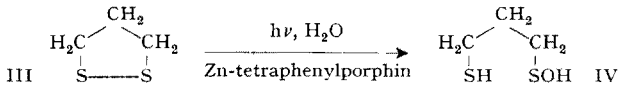
atom of a thio ether hydrate I to an sp^3d^2 hybrid orbital II[1] to form the same dihydrosulfoxide oxidation state[11],

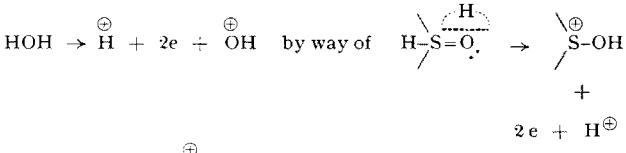


which is obtained by oxidation of the cyclic thiazolidine form of acyl cysteine in formic acid and hydrogen peroxide[1]. A similar mechanism has been reported for methionine and water as a photochemical electron donating system and methylene blue[12] and riboflavin as electron acceptors[13,14]. Such a cytochrome dihydrosulfoxide might be one of the 'energetic or reduced cofactors acting as carriers of hydrogen and energy from the light reactions to the carbon reduction cycle' as was postulated by CALVIN and BASSHAM[15].

CALVIN et al.[16] have observed photooxidation of 1,2-dithiolane III to the monosulfoxide IV in the presence of zinc tetraphenyl porphin. They proposed a fission of the disulfide group by light and addition of water.



If the role of the sulfur function in cytochrome follows the glutathione model it would serve to separate H_2O into



in which the $\overset{\oplus}{>}$S–OH group is the 'unknown electron acceptor' (FRUTON and SIMMONDS[17]), 'hole' or oxidizing agent as it was depicted by CALVIN and BASSHAM[15]. In aqueous solution it would play the same role as the H_2O_2 appearing with catalases and peroxidases. (It is assumed that in plants these enzymes catalize the oxidation of metabolites.)

The following considerations suggest a mechanism for a possible linkage of the photolysis of H_2O with the CO_2 fixation.

Besides the ability of sulfides to react with water under proper conditions to form a dihydrosulfoxide, a methylene group or methine group adjacent to sulfur is activated to form a carbanion. The best investigated methine group is the CH at the position number 2 of the thiazole ring of TPP (thiamine pyrophosphate)[18,19]. BRESLOW[20,21] introduced the idea of the possible involvement of *d*-hybrid orbitals of the sulfur in the thiazole ring of TPP to explain the activation of the hydrogen on the TPP thiazol ring position number 2. BRESLOW emphasized the significance of resonance stabilization of the intermediate carbanion. Hydrogen on the α-carbon atom of a sulfoxonium derivative[22], can be deuterated as rapidly in the absence of base as can the thiazole ring[20,23].

The biological role of this carbanion is to form bonds with carbonyl compounds like pyruvic acid[18,19].

WHITE and INGRAHAM[24] recently invoked the concept of the 2-thiazolium carbanion, but they did not disc uss the implication of the sulfur bond orbital hybridization.

The *in vitro* acylation of an α-C atom of dimethyl sulfoxide was demonstrated by HORNER and KAISER[25], who proposed an intermediate sulfoxide association. There appears some analogy in the stabilization of a carbanion in TPP and in sulfoxides.

Carboxydismutase has been described as a carboxylating enzyme in the photosynthesis[26]. This enzyme would transfer the CO_2 to ribulose-1, 5-diphosphate[27]. BASSHAM[28] suspects 'some organization of the various enzymes associated with the structure of the chloroplast'. One is led to suspect a mechanism for CO_2 fixation similar to that established for the pyruvic acid TPP addition[18,19].

It is but a small, additional speculative step to involve the sulfur function linking the vinyl group of the porphyrin in cytochrome I to the protein in the fixation and transfer of CO_2 and other carbonyl compounds. The side chain C atom between porphyrin and sulfur in this molecule is in the same activated position as the C_2 in the thiazole due to the ability of sulfur to assume a *d*-hybridized S-outer shell. We see that porphyrin is the ideal resonator for a conjugated carbanion. It is therefore a potential primary fixation point for CO_2 to give a cytochrome CO_2 adduct similar to the chloroplast CO_2 adduct suggested by WARBURG[29]. It could as well be instrumental in attaching and transferring any other carbonyl group observed to react with thiamine.

In the model the reducing hydrogen is available at the neigboring thio ether hydrate which can be expanded to the dihydrosulfoxide[1] and conforms with the concept of a reductive carboxylation[28].

Zusammenfassung. Die Dihydrosulfoxidgruppe wird als potentielle Wirkungsgruppe für die Photolyse von Wasser zur Diskussion gestellt. Die Möglichkeiten der Elektronenübertragung von Cytochromdihydrosulfoxid werden erwogen.

G. E. UTZINGER[29]

Department of Pharmaceutical Chemistry, The University of California, School of Pharmacy, San Francisco (California, U.S.A.), November 26, 1962.

[11] Perhaps, in a formal way, it may more correctly be described as a new co-ordination state of the hydrate.

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