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GLUTATHIONE PEROXIDASE : REDOX CHEMISTRY OF ACTIVE SITE MODEL PEPTIDES

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Abstract: Di and tetrapeptides (Sec-Gly and Sec-Gly-Thr-Thr) mimicking the active site of glutathione peroxidase catalyse the reduction of H_2O_2 in the presence of thiols, via the formation of a cyclic selenamide.

The reduction of hydroperoxides by the selenoenzyme glutathione peroxidase has been claimed to be a major protective system of mammalian cells against oxidative damage [1]. The protein is characterized by an active site including a selenocysteine residue (Sec) which is essential for the catalytic activity [2].
The mechanism by which the enzyme works in the presence of glutathione (GSH) is still debated. According to X-ray crystal structural and biochemical data [2], it has been postulated that the active site selenolate (Enz-Se^-) would react with hydroperoxides to be oxidised either to versatile selenenic acid (Enz-SeOH) or to seleninic acid (Enz-Se(O)OH) when peroxide concentration is high. The selenenic acid would have only a transient existence and would undergo immediate reduction by excess of GSH normally present in the cell. Thus the main reaction would shuttle between the selenolate and the selenenic acid states. More recently another intermediate in the catalytic cycle has been proposed by Jasperse and Reich using simple synthetic models [3]. A cyclic selenenamide was suggested to be the oxidised form of the enzyme
This implied the formation of an unusual isoselenazolidin-3-one which can be related to benzisoselenazolines with glutathione peroxidase-like activity, previously described [1c,4], e.g. Ebselen.

The present report deals with a new approach to the problem, starting from the di and tetrapeptide analogues of the active site namely Sec-Gly and Sec-Gly-Thr-Thr. In the preparation of selenopeptides , we have introduced the selenic group at the last step by nucleophilic displacement of the O-tosyl group of a protected serine using the sodium salt of the benzylselenol (Bzl-Se^-) [5]. The protected dipeptides Z-Ser(Tos)-Gly-OEt **1** and tetrapeptide Z-Ser(Tos)-Gly-Thr(Bzl)-Thr(Bzl)-OBzl **2** [6] gave respectively Z-Sec(Bzl)-Gly-OEt **3** and Z-Sec(Bzl)-Gly-Thr(Bzl)-Thr(Bzl)-OBzl **4** upon treatment with sodium benzyl selenolate [7]. Reaction of the protected selenopeptides **3** and **4** with bromine [8] yielded the selenenyl bromides Z-Sec(Br)-Gly-OEt **5** and Z-Sec(Br)-Gly-Thr(Bzl)-Thr(Bzl)-OBzl **6** which were easily converted either to diselenides **7** and **8** by the action of triethylamine under aerobic conditions or to the cyclic selenenamides **9** and **10** under argon. Compounds **1-8** have been fully

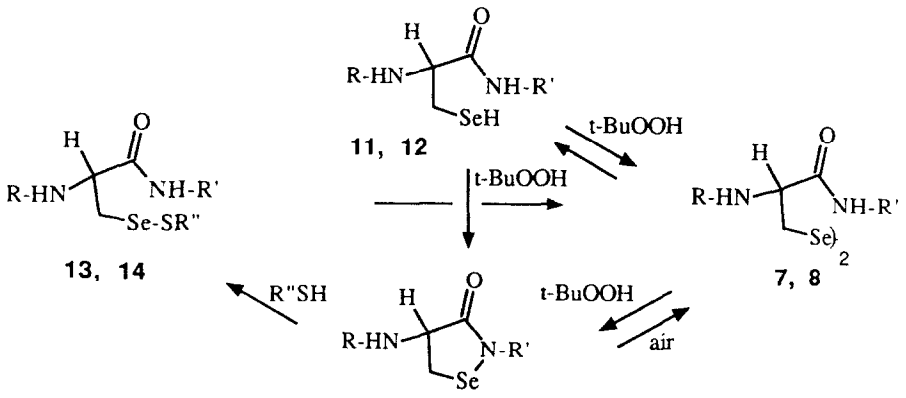
characterized by ^{1}H and ^{77}Se NMR and FAB mass spectroscopy [6]. The isoselenazolidines **9** and **10** were unstable : in the presence of air, they gave the diselenides **7** and **8**. The formation of the heterocycle has been evidenced by ^{1}H NMR by diseappearance of the NH(Gly) signal, a downfield shift of the αCH(Sec) at 4.70 ppm, βCH_2(Sec) at 3.67 and 3.96 ppm and CH_2(Gly) at 4.05 ppm. These features were in accordance with previous models of Jasperse [3,8,9]. The ^{77}Se NMR were in keeping with the cyclic selenenamide assigned structure with a chemical shift of 842 ppm for **9** and 848 ppm for **10**.

Reduction of the isoselenazolidines

Reduction of the isoselenazolidines **9** (or **10**) with mercapto acetic acid in the presence of trifluoroacetic acid gave a mixture of diselenides **7** (or **8**) and selenosulfides **13** (or **14**) ($\delta^{77}Se$: 405 ppm) under argon (the selenosulfides slowly undergo a cleavage reaction to selenides as previously described by Fischer [10]). In the presence of air, only diselenides were obtained.

Oxidation of the selenol

The selenol Z-Sec-Gly-OEt **11** ($\delta^{77}Se$: -71 ppm) and Z-Sec-Gly-Thr(Bzl)-Thr(Bzl)-OBzl ($\delta^{77}Se$: -75 ppm) **12** were obtained via reduction by sodium borohydride of the respective diselenides and were particularly stable in chloroformic solution under uncontrolled atmosphere. Oxidation of the selenol **11** with t-butylperoxide in the presence of mercaptoacetic acid gave a mixture of selenenamide **9** and diselenide **7**. Then the ^{77}Se NMR selenenamide signal diseappeared rapidly to give selenosulfide **13**. Three hours after the addition, only the diselenide **7** was observed. It was noteworthy that the selenol **11** and diselenide **7** did not react with mercaptoacetic acid under neutral conditions.



Scheme I

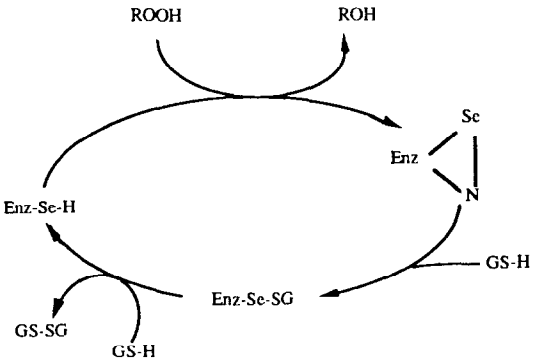
Scheme I summarizes the redox results. Oxidation of selenol **11** did not lead to seleninamide as shown by Reich and Jasperse [3] nor to selenenic acid [2a] but gave cyclic selenenamide either directly from selenol or from diselenide as intermediate. The selenenamide seems to be the oxidative form of the enzyme. These results suggest that the selenenamide is the oxidised form of the enzyme. It was thus interesting to test these peptides for glutathione peroxidase activity.

Glutathione peroxidase activity

Glutathione peroxidase activity of the diselenides **7** and **8** which can be considered as stable precursors of the cyclic selenamide, was determined according to a modification of the method of Wendel [11]. Hydrogen peroxide was used as the substrate in the presence of GSH. Gluthathione reductase was used to reduce the oxidized GSH with NADPH as cofactor. In this test the decreased in NADPH concentration was followed spectrometrically at 366 nm and was considered as a measure of GSH peroxidase activity. Under these conditions glutathione peroxidase 0.1 U was used as standard reference.

The diselenides **7** and **8**, exhibited a higher glutathione peroxidase activity (0.67 and 0.71 relative GSH peroxidase activity) than Ebselen (0.33 relative GSH peroxidase activity). Since this drug has been reported to possess very promising anti-inflammatory properties and strong glutathione peroxidase-like activity [1c,4], the selenopeptides **3** and **4** may be considered as good models for the active site of glutathione peroxidase.

We have shown that active site model peptides catalyze the hydrogen peroxide reduction in the presence of glutathione with much higher chemical efficiency that does a model selenoheterocyclic compound. Since the enzymatic reaction is mechanistically similar to the redox chemistry of glutathione peroxidase, a pathway consistent with the observations reported here can be suggested (Scheme II).



Scheme II

In the catalytic cycle of the enzyme, the diselenide form can be definitely discarded owing to steric constraints due to the protein bulk. The turnover reaction may reasonably proceed via a nucleophilic attack of the glutathione thiolate group on the cyclic selenamide formed after reaction with the peroxides as suggested by Reich and Jasperse [3]. The formation of a transient selenenic form cannot be excluded, but consistent with the high unstability of alkyl selenenic acids, no observable amounts were found here. These results could constitute the basis for the rational design of selenium-based antioxidants and efficient redox catalysts.

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