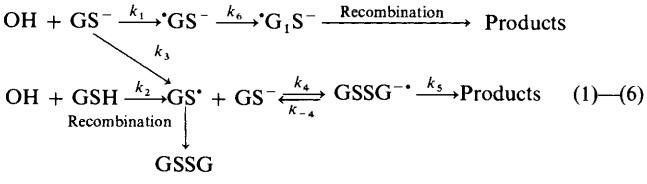
Formation of Reducing Radicals on Radiolysis of Glutathione and Some Related Compounds in Aqueous Solution

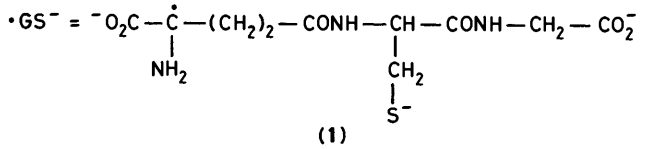
Trygve E. Eriksen * **and Gunilla Fransson**
Department of Nuclear Chemistry, The Royal Institute of Technology, Fack, S-100 44 Stockholm 70

The 'OH-induced oxidation of glutathione in basic aqueous solution has been found to produce the sulphur-centred radical GS' ($GSSG^{-\bullet}$) and two reducing radicals. Decarboxylation, considered to be initiated by 'OH radical addition to the nitrogen of the amino group on the γ-glutamyl unit, was found to be effective for methylated glutathione (GSMe) and ophthalmic acid (GMe) but of minor importance for glutathione. The *G*-values for the strongly reducing α-amino radicals formed on decarboxylation were found to be 3.3, 3.3, and 0.5 for GSMe, GMe, and GSH, respectively, at pH 10.5. The formation of an additional strongly reducing radical with $G = 2.2$ at pH 10.5 was demonstrated by measuring the electron-transfer to *p*-nitroacetophenone (PNAP). This radical is not formed from GSMe or GMe. We suggest that the underlying mechanism for the formation of this species is based on pH-dependent conformational changes of glutathione followed by 'OH addition to the cysteine sulphur, or hydrogen abstraction from the CH_2 group in the α-position to the sulphur leading to the formation of a reducing carbon-centred radical.

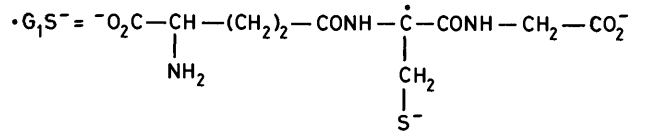
In a recent study on the 'OH radical-induced oxidation of glutathione[1] (γ-glutamylcysteinylglycine, GSH) in alkaline solution it was found that in addition to the sulphur-centred radical GS' ($GSSG^{-\bullet}$) produced by electron-transfer or hydrogen abstraction from GS^- (GSH) at least one other radical is formed. Based on the pH-dependence of the radical yields and published electron spin resonance studies[2,3] on amino acids at pH 13 it was suggested that the second radical was formed by hydrogen abstraction from the carbon atom at the α-position to the amino group on the glutamyl unit and transferred intramolecularly to a carbon-centred radical in the β-position to the sulphydryl group of the cysteine residue according to the Scheme where the structures (**1**) and (**2**) were tentatively assigned to the radicals $^{\bullet}GS^-$ and $^{\bullet}G_1S^-$ respectively.



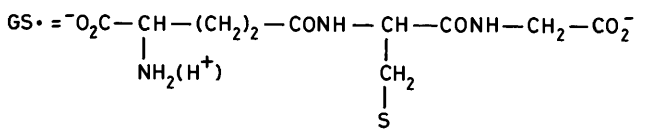
Scheme.



(**1**)



(**2**)



(**3**)

The thiol radical (**3**) is stabilized by formation of a sulphur–sulphur-bonded complex $GSSG^{-\bullet}$ (**4**) with the thiolate ion.

$$-CH_2S^{\therefore(-)}SCH_2- \quad (4)$$

This radical ion exhibits a characteristic absorption with $\lambda_{max.}$ *ca.* 420 nm.[1]

Whereas GS' is an oxidizing species[4,5] $GSSG^{-\bullet}$ is a reducing species; thus pH and glutathione concentration will have a marked effect on the primary GS' radical. According to the proposed structure, $^{\bullet}GS^-$ is an 'α-amino' radical and should therefore be a strong reductant in accordance with data from e.s.r.,[6] pulse radiolysis,[7,8] and photochemical[9] studies.

In recent studies Asmus[10] and co-workers have demonstrated that 'OH radical-induced oxidation of the sulphur-containing amino acid methionine leads to quantitative decarboxylation and the formation of an α-amino radical. The reaction mechanism proposed is based on intramolecular oxidation of the amino group by the initially oxidized sulphur.[11,12] The 'OH radical-induced decarboxylation of amino acids with an unprotonated amino group, *i.e.* in alkaline solution, was also found to be very effective.[13]

In the present paper we give a more detailed report on some properties of radicals formed in the reaction between 'OH radicals and glutathione in basic aqueous solution. For comparison ophthalmic acid (GMe) and *S*-methylglutathione (GSMe), having the same peptide backbone and terminal carboxylic acid and amino groups but with the cysteine SH group being replaced by Me or SMe groups respectively, have also been studied.

Experimental

p-Nitroacetophenone (PNAP), GSH (pa-quality, Fluka), ophthalmic acid (res. grade, Serva), *S*-methylglutathione (Sigma), and all other chemicals (p.a. Merck) were used as received. The solutions, prepared from water which was doubly distilled in quartz, were made oxygen-free by purging with Ar (AGA-SR quality < 1 p.p.m. O_2) or saturated with N_2O (AGA). Precautions were taken to minimize air oxidation of the glutathione as well as decomposition at high pH. The buffer solutions were deoxygenated by Ar before adding the GSH. The

pH was adjusted with NaOH or $HClO_4$ and the solutions were N_2O-saturated immediately before use. The N_2O converts the reducing hydrated electrons into $^{\bullet}OH$ radicals *via* $e^-_{aq} + N_2O \longrightarrow N_2 + OH^- + {}^{\bullet}OH$.

As e^-_{aq}, $^{\bullet}OH$, and $^{\bullet}H$ are formed with *G*-values of 2.8, 2.8, and 0.6 respectively, at least 90% of the reactive primary species are $^{\bullet}OH$ radicals. (The *G*-value is defined as the number of molecules changed per 100 eV energy absorbed.) All experiments were carried out at ambient temperature (20 ± 1 °C).

The carbon dioxide yields of γ-irradiated solutions (dose rate 43 Gy min^{-1}) were determined by a gas chromatographic technique.[14] Irradiated N_2O-saturated aqueous solutions of glutathione were adjusted to pH 10.5 with NaOH to convert CO_2 into the anionic form according to the equilibrium

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \ (pK_a\ 6.37)$$

and N_2O was removed by purging with Ar. The solutions were thereafter acidified to pH <3 and the CO_2 concentration in the gas phase (Ar) in equilibrium with the acidified solutions was measured with an AGA-Argograph equipped with a 180 cm × 0.3125 cm, 150—200 mesh Poropak Q column.

Calibration was carried out by introducing known amounts of CO_3^{2-} or CO_2 into unirradiated solutions and following the procedure described above. All data were corrected for the solubility of CO_2.

The pulse radiolysis experiments were carried out by applying 0.2—1 μs long electron pulses from a 7 MeV microtron accelerator. The dose/pulse (5—20 Gy) was measured with a secondary emission chamber, which was previously calibrated using a N_2O-saturated aqueous 10^{-2}M-KSCN solution as standard chemical dosimeter with $G\varepsilon(CNS)_2^{-\bullet} = 4.64 \times 10^4$ mol^{-1} dm^3 cm^{-1} at 500 nm.[15] Details of the pulse radiolysis set-up are given elsewhere.[16] Data storage and analysis were based on a Biomation 8 100 transient recorder and a PDP11/40 computer. The signal/noise ratio of small signals was improved by superposition of several signals.

Results and Discussion

The transient spectrum on pulse radiolysis of N_2O-saturated aqueous alkaline solutions of glutathione is dominated by a strong visible absorption ($\lambda_{max.}$ 420 nm, ε 8 × 10^3 mol^{-1} dm^3 cm^{-1}) unambiguously assigned to the $GSSG^{-\bullet}$ radical ion,[1] and an absorption band with absorption maximum at about 270 nm. The latter absorption band is somewhat distorted due to the $GS^{\bullet}$ absorption ($\lambda_{max.}$ 320 nm). The 420 nm absorption band assignable to the $GSSG^{-\bullet}$ radical anion is not present in the transient spectra recorded after pulse irradiation of N_2O-saturated solutions of GSMe and GMe at pH 10.5 and as can be seen (Figure 1) the time dependence of the transient spectra at λ < 350 nm are entirely different. The decay of the 270 nm absorption band in irradiated GSMe and GMe solutions was found to follow second-order kinetics. In the case of GSH the exponential build-up of an absorption band with continuously increasing intensity from 320 nm towards u.v. wavelengths is followed by a slow second-order decay (Table 1). The first-order build-up is not concomitant with the decay of the $GSSG^{-\bullet}$ absorption band at 420 nm and has been ascribed by Sjöberg to the $^{\bullet}GS^- \longrightarrow {}^{\bullet}G_1S$ transformation defined in the Scheme.

The optical absorbance of $GSSG^{-\bullet}$ at 420 nm measured directly after the electron pulse and the maximum absorption at 280 nm following the build-up have been plotted *versus* pH in Figure 2. The absorbance at 420 nm has been corrected with respect to the equilibrium

$$GS^{\bullet} + GS^- \overset{K_4}{\rightleftharpoons} GSSG^{-\bullet}$$

using the expression

$$GS^{\bullet}_{tot} = GSSG^{-\bullet}\,[1 + (1 + H^+/K_{SH})/K_4 \cdot GSH_{tot}] \quad (7)$$

where K_{SH} is the ionization constant of the thiol group (pK_{SH} 9.2) and the stability constant K_4 4.4 × 10^3 mol dm^{-3}. Assuming $\varepsilon_{GSSG^{-\bullet}}$ to be 8 × 10^3 mol^{-1} dm^3 cm^{-1} at 420 nm it can be seen that $G_{GSSG^{-\bullet}} = 6.2$ for pH <7.8. Thus both $^{\bullet}H$ and $^{\bullet}OH$ react quantitatively with the protonated thiol group, yielding the $GS^{\bullet}$ radical. In the pH range 8—10.5 the 420 nm absorption decreases and the 280 nm absorption increases.

The increase in the absorbance at 280 nm in this pH range has been attributed to the increased reactivity of the amino-containing group due to deprotonation of the amino group (pK_a 9.5).

The decrease in the 420 nm absorption in the pH range 8—

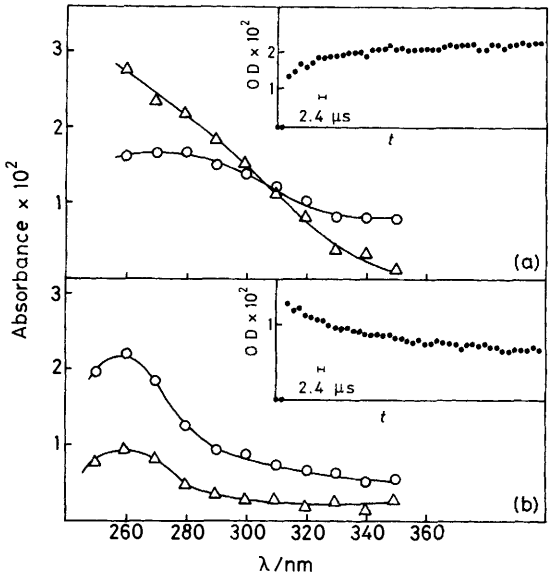


Figure 1. Transient spectra recorded in pulse-irradiated, N_2O-saturated solutions at pH 10.5 of (a) 5 × 10^{-3}M-GSH, ○ end of pulse, △ 300 μs after pulse; (b) 3.5 × 10^{-3}M-GSMe•Me, ○ end of pulse, △ 300 μs after pulse. Dose 100 Gy. Inserts, traces recorded at 280 nm

Table 1. Kinetic data for the 280 nm absorption, product yields at pH = 10.5

	GSH	GSMe	GMe	Glycine
G ε at 280 nm (mol^{-1} dm^3 cm^{-1})	Initial: 16 600 max: 20 000	Initial = max: 12 600	Initial = max: 13 200	
Build-up	$k = (2 \pm 0.3) \times 10^4$			
Decay $2k/\varepsilon$	6.3 × 10^3	(1.3 ± 0.1) × 10^5	(1.0 ± 0.1) × 10^5	
$G(PNAP^{-\bullet})$	2.8	3.5	3.0	
$G(CO_2)$	0.5 ± 0.05	3.3 ± 0.3	3.3 ± 0.3	3.2 ± 0.2

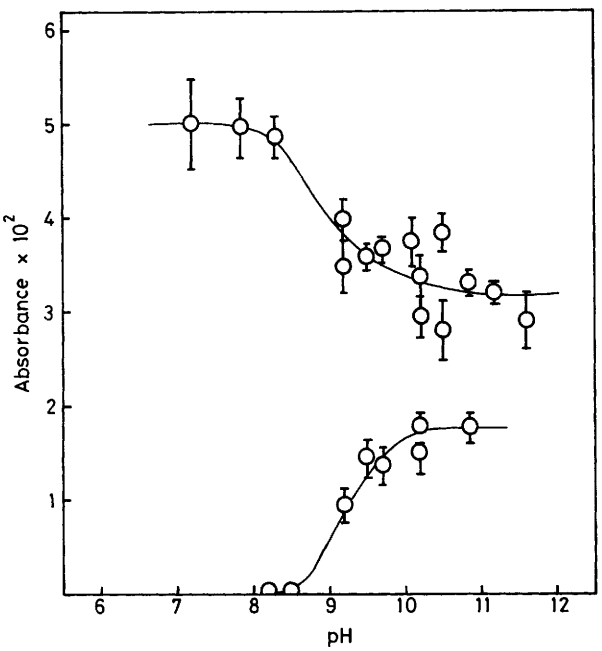


Figure 2. Changes in optical absorption recorded in pulse-irradiated, N_2O-saturated solutions of 5×10^{-3} mol dm^{-3} GSH as a function of pH (dose 10 Gy). Upper curve recorded at 420 mm and corrected for the $GS^{\bullet} + GS^- \rightleftharpoons GSSG^{-\bullet}$ equilibrium. Lower curve recorded at 280 nm

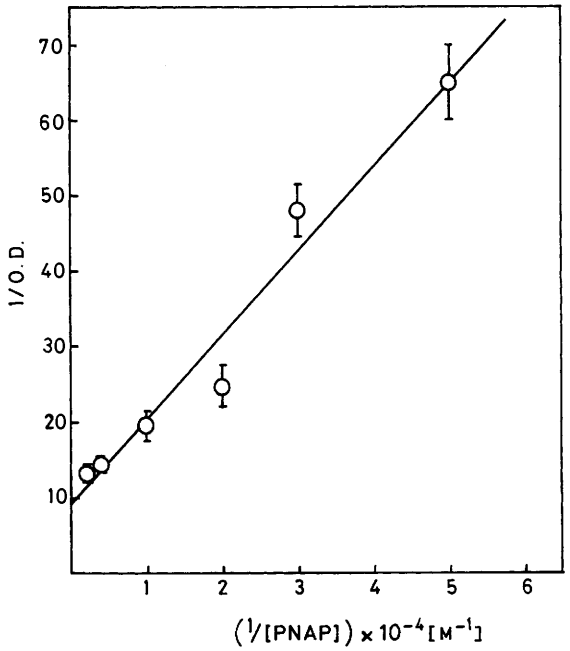


Figure 3. Optical absorption recorded at 350 nm after pulse irradiation of N_2O-saturated solutions at pH 8 containing 10^{-2} mol dm^{-3} GSH and varying PNAP concentrations

10.5 corresponds to a decrease in the $G(GSSG^{-\bullet})$ value from 6.2 to 4. If it is assumed that the hydrogen atom reacts slowly or not at all with the deprotonated thiol group, this means that the 420 nm absorption accounts for *ca.* 70% of the initially formed $^{\bullet}OH$ radicals. If all $^{\bullet}H$ and 30% of the $^{\bullet}OH$ radicals produce the radical $^{\bullet}GS^-$ at pH 10.5 the absorbance at 280 nm corresponds to a *G*-value of 2.2.

The rate constants for reactions (1)—(3) were determined in a competition kinetic study using CNS^- as reference.

$$OH + CNS^- \xrightarrow{k_7} CNS^{\bullet} \qquad (8i)$$

$$CNS^{\bullet} + CNS^- \rightleftharpoons (CNS)_2^{-\bullet} \qquad (8ii)$$

The concentration of $(CNS)_2^{-\bullet}$ at differing [GSH]/[CNS^-] ratios was determined by measuring the optical absorption at 540 nm. As both $(SCN)_2^{-\bullet}$ and $GSSG^{-\bullet}$ absorb at this wavelength the rate constant for the reaction between $^{\bullet}OH$ and $(GSH)_{tot}$ was calculated using the expression

$$\frac{(C_0\varepsilon_{(CNS)_2^{-\bullet}} - OD_t)}{(OD_t - C_0\varepsilon_{GSSG^{-\bullet}})} = \frac{k_1[GSH]_{tot}}{k_7[CNS^-]} \qquad (9)$$

where OD_t is the optical density at 540 nm, and C_0 is the initial concentration of $^{\bullet}OH$ radicals. The overall rate constants were calculated assuming $\varepsilon_{(CNS)_2^{-\bullet}}$ and $\varepsilon_{GSSG^{-\bullet}}$ to be 4 200 and 2 100 mol^{-1} dm^3 cm^{-1} respectively at 540 nm[2] and k_7 1.2×10^{10} mol^{-1} dm^3 s^{-1}.[15] The rate constants obtained at pH 7.8 and 10.6 are given in Table 2 together with rate constants for GSMe and GMe at pH 4 and 10.6 determined in corresponding experiments.

In order to obtain information on the assumed reducing properties of the radical ($^{\bullet}GS^-$) the reaction with *p*-nitroacetophenone (PNAP) was studied at pH 8 and 10.5. The reactions assumed to take place were

Table 2. Kinetic parameters for the $^{\bullet}OH$ reactions

Substance	pH	Rate constants (overall) mol^{-1} dm^3 s^{-1}
GSH	7.8	$(1.4 \pm 0.1) \times 10^{10}$
GSH	10.6	$(4.4 \pm 0.5) \times 10^{10}$
GSMe	4	$(0.9 \pm 0.1) \times 10^9$
GSMe	10.6	$(5.2 \pm 0.1) \times 10^9$
GMe	4	$(2.1 \pm 0.05) \times 10^9$
GMe	10.6	$(6.9 \pm 0.5) \times 10^9$

$$GSSG^{-\bullet} + PNAP \xrightarrow{k_8} PNAP^{-\bullet} + GSSG \qquad (10)$$

$$\downarrow k_5$$

Products

$$^{\bullet}GS^- + PNAP \xrightarrow{k_9} PNAP^{-\bullet} \qquad (11)$$

$$\downarrow k_3$$

$$^{\bullet}G_1S^-$$

At pH <8 no $^{\bullet}GS^-$ is formed as can be seen from Figure 2, and the rate constant k_8 was therefore determined by measuring the optical absorption of the $PNAP^{-\bullet}$ radical anion at 350 nm (ε 17 600 mol^{-1} dm^3 cm^{-1})[17] in N_2O-saturated solutions containing 10^{-2}M-GSH and varying concentrations of PNAP.

The k_8/k_5 ratio and $GSSG^{-\bullet}$ yield were calculated from the plot of $PNAP^{-\bullet}$ absorptions *vs.* 1/(PNAP) in Figure 3 to be $(8 \pm 0.6) \times 10^3$ and 6.2, respectively. The value of $G_{GSSG^{-\bullet}}$ is in excellent agreement with the data obtained by measuring the absorbance at 420 nm. Baker and co-workers in a recent work[18] found $\varepsilon_{GSSG^{-\bullet}}$ at 420 nm and K_4 to be 6.3×10^3 mol^{-1} dm^3 cm^{-1} and 1.67×10^3 mol^{-1} dm^3 respectively. The $G_{GSSG^{-\bullet}}$ value calculated, when applying these constants to our experimental data is, however, far higher than the corresponding $G_{GSSG^{-\bullet}}$ value obtained in our PNAP experiments. In our

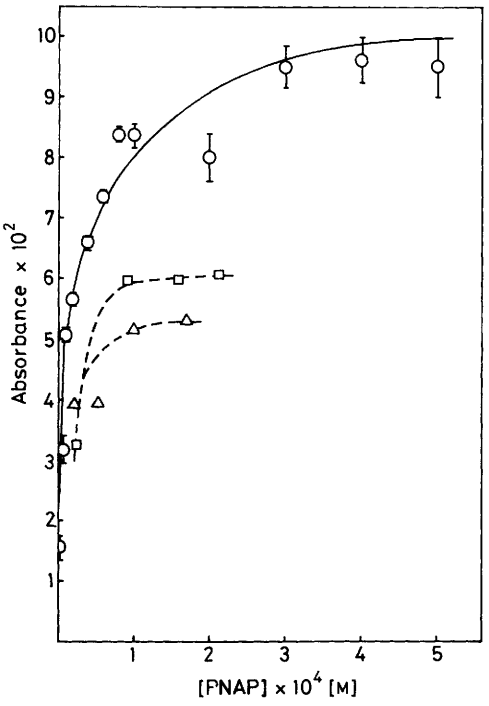


Figure 4. Optical absorption recorded at 350 nm after pulse irradiation of N_2O-saturated solutions at pH 10.5 containing 10^{-2} mol dm^{-3} GSH^- (○), 2×10^{-3} mol dm^{-3} GSMe (□), 3.5—10^{-3} mol dm^{-3} GMe (△) and varying concentrations of PNAP. The full line was computed using the parameters $G_{GSSG^{-\cdot}} = 3.7$, $G_{\cdot GS} = 2.8$, $k_8/k_5 = 8 \times 10^3$ and $k_9/k_6 = 4 \times 10^5$

opinion, therefore, the molar absorbance and equilibrium constants given by Baker *et al.* are too low.

At pH = 10.5 $PNAP^{-\cdot}$ absorbance develops at a much lower PNAP concentration than at pH = 8 and the experimental data cannot be accommodated by a single-electron-transfer reaction. Taking into account reactions (10) and (11) above, the $^{D}PNAP^{-\cdot}$ absorption is given by the equation (12).

$$\text{O.D.}_{PNAP^{-\cdot}} = (\varepsilon_{PNAP^{-\cdot}}) \times \text{Dose} \times 10^6 \times \left[\left(\frac{k_8[PNAP]}{k_8[PNAP] + k_5}\right)G_{GSSG^{-\cdot}} + \left(\frac{k_9[PNAP]}{k_9[PNAP] + k_6}\right)G_{\cdot GS^-}\right] \quad (12)$$

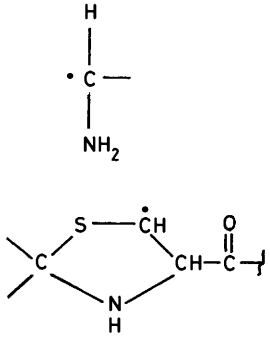
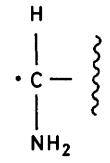
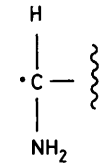
The best fit of this equation to the experimental data plotted in Figure 4 was obtained using the parameters

$$G_{GSSG^{-\cdot}} = 3.7\ G_{\cdot GS^-} = 2.8;\ k_8/k_5 = (8 \pm 0.6) \times 10^3 \text{ and } k_9/k_6 = (4 \pm 1) \times 10^5$$

The $GSSG^{-\cdot}$ yield obtained in the PNAP experiments is in fairly good agreement with the yield obtained from measurements of the optical absorption of $GSSG^{-\cdot}$ at 420 nm and it is evident from the plot in Figure 4 that a stronger reducing species than the radical anion $GSSG^{-\cdot}$ is formed with a *G*-value of 2.8.

Corresponding experiments were carried out at pH 10.5 with GSMe and GMe and the data are plotted in Figure 4. The rate constants were calculated from kinetic traces at PNAP $> 2 \times 10^{-4}$ mol dm^{-3}. The rate constants and $G(PNAP^{-\cdot})$ values are summarized in Tables 1 and 3.

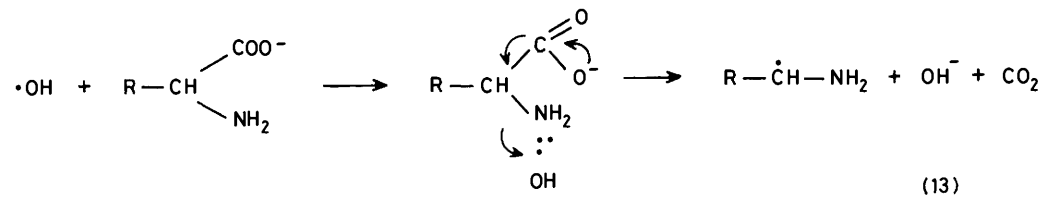
Table 3. Kinetic data for formation of the $PNAP^{-\cdot}$ radical anion

Substance	pH	Suggested radical	Rate constant mol^{-1} dm^3
GSH	8.0	$GSSG^-$	$(8 \pm 0.6) \times 10^7$
	10.5		$(8 \pm 1) \times 10^9$
GSMe	10.5		$(3.0 \pm 0.1) \times 10^9$
GMe	10.5		$(1.8 \pm 0.2) \times 10^9$

The results from CO_2 analysis of γ-irradiated N_2O-saturated solutions of GMe, GSMe, and GSH at pH 10.5 are plotted in Figure 5 and the *G*-values are given in Table 1. With the analogues approximately 50% of the yield of $^{\cdot}OH$ radicals is accounted for by the decarboxylation reaction. The remaining $^{\cdot}OH$ radicals react mainly by hydrogen abstraction from, as an example, the carbon adjacent to the peptide NH function. Whereas the yields of decarboxylation and $PNAP^{-\cdot}$ formation are the same for GSMe and GMe there is a marked difference in the case of GSH, the CO_2 yield being much smaller than the yield of $PNAP^{-\cdot}$. It is therefore evident that at least two different strongly reducing species are formed on radiolysis of N_2O-saturated basic aqueous solutions of glutathione.

The CO_2 yields obtained on γ-radiolysis of N_2O-saturated solutions of glutathione at varying pH are plotted in Figure 6. From the optical measurements of the $GSSG^{-\cdot}$ absorption and the $PNAP^{-\cdot}$ absorption at 540 nm the total yield of strongly reducing species produced on pulse radiolysis of N_2O-saturated solutions of GSH at pH 10.6 corresponds to a *G*-value of 2.5 ± 0.3. From the plots of optical absorbance at 290 nm (Figure 1) and $G(CO_2)$ *vs.* pH the yields of $^{\cdot}GS^-$ have been calculated and plotted in Figure 6. As can be seen the formation of the strongly reducing species $^{\cdot}GS^-$ and the reaction leading to decarboxylation display similar pH-dependence.

The $^{\cdot}OH$ radical-induced decarboxylation of amino acids having the amino group deprotonated has recently been discussed by Mönig *et al.*[13] and the following mechanism has been suggested.



(13)

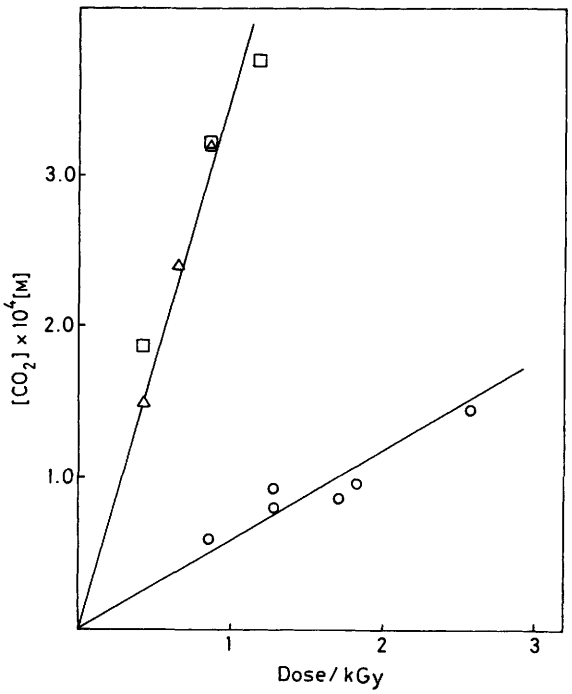


Figure 5. Total yields of CO_2 after γ-irradiation of N_2O-saturated solutions at pH 10.5 containing 10^{-2} mol dm^{-3} GSH (○), 3.5×10^{-3} mol dm^{-3} GSMe (□), and 4.5×10^{-3} mol dm^{-3} GMe (△) as a function of the dose

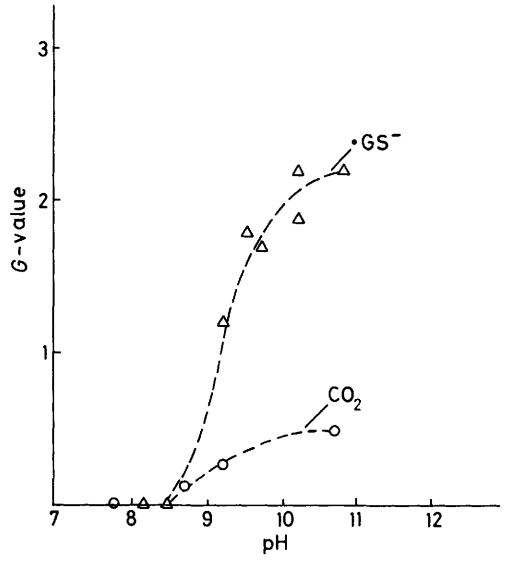
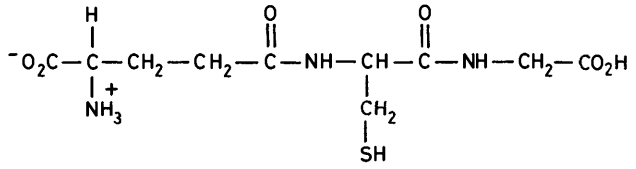


Figure 6. *G*-Values for the formation of CO_2 and $^{\bullet}GS^-$ in N_2O-saturated aqueous solutions of 5×10^{-3} mol dm^{-3} glutathione (GSH) plotted *vs.* pH

i.e. addition of •OH to the free-electron pair at the nitrogen followed by spontaneous decarboxylation and the formation of an α-amino alkyl radical.

In a recent paper Amus and Hiller[10] have shown that the electron-transfer from the α-amino alkyl radical formed by •OH oxidation of methionine to PNAP is a fast process $k = (3.9 \pm 0.4) \times 10^9$ mol^{-1} dm^3 s^{-1}. Neglecting the small decarboxylation yield ($G \simeq 0.5$) and assuming $k_6 = 2 \times 10^4$ s^{-1} we estimate the rate constant for the electron-transfer from $^{\bullet}GS^-$ to PNAP to be $k_9 \sim 8 \times 10^9$ mol^{-1} dm^3 s^{-1}, *i.e.* of the same order of magnitude as obtained by Asmus for an α-amino alkyl radical.

The generally accepted formula for glutathione is



Glutathione is thus really a diamide having two carboxylic acid groups, one thiol group and one amino group with pK_a values 2.5, 3.7, 9.2, and 9.5[18] respectively. In the following the protonation state of the amino group will be denoted in brackets. As can be seen from Table 1 the •OH radical reacts much more slowly with $[NH_2]$GMe and $[NH_2]$GSMe than with $[NH_2]GS^-$. All three molecules have the same peptide backbone structure as well as terminal carboxylic acid and amino groups. It therefore seems reasonable to assume that the reactivity of the deprotonated amino group should be nearly the same for all three molecules. Based on the assumption of straightforward kinetic competition for the •OH radical, the $G(CO_2)$-value and the rate constants for •OH attack on species $[NH_2]GS^-$, $[NH_2]$GMe, and $[NH_2]$GSMe in Table 1 the yield of CO_2 is predicted to be

$$G(CO_2) \sim 3.3 \frac{0.5\ [6.9 \pm 5.2] \times 10^9}{4.4 \times 10^{10}} \sim 0.45$$

i.e. in good agreement with the $G(CO_2)$-value obtained in γ-radiolysis.

The hydrogen-abstraction reaction suggested by Sjöberg for the formation of the $^{\bullet}GS^-$ radical cannot easily be envisaged. Our experiment data clearly indicate that some pH-dependent conformational changes take place and that the SH/S^- group of the cysteine residue is involved in the formation of the reducing radical $^{\bullet}GS^-$.

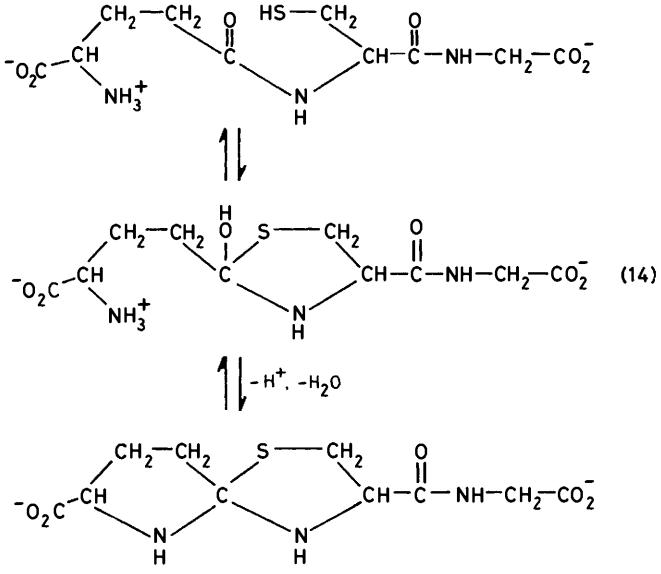
Experimental data on the glutathione structure are rather scarce. The crystal structure determined by Wright[19] and n.m.r.[20] studies of glutathione derivatives give no information on the partially or fully dissociated molecule in solution. Quite differing conclusions have been reached from n.m.r. studies of glutathione in aqueous solution. Zenin *et al.*[21] conclude that there is interaction between the glutamyl NH_3^+ and the glycyl CO_2^- groups as well as between the glutamyl CO_2^- group and the peptide NH groups.

According to Fujiwara *et al.*[22] the preferred conformation of the fully dissociated molecule is, however, one in which the carboxy and amino groups are at a distance far from the

peptide backbone. It is also concluded that changes in the functional groups of glutamic acid have great influence on the preferred conformation, which is also supported by theoretical calculations.[23] The SH/S^- is in all cases far from the peptide backbone.

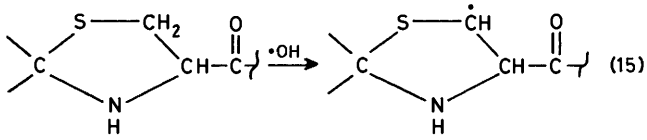
The acid–base equilibria of glutathione have been studied by ^{13}C n.m.r.[24,25] The dissociation constants obtained are in fair agreement, but there are some differences in the assignment of chemical shifts. The dissociation of the amino group on the γ-glutamyl unit according to Jung *et al.*[24] causes a considerable ^{13}C shift (δ 8.6 p.p.m.) of the α-CO_2 carbon. A corresponding shift is, however, assigned to the glutamyl γ-C=O carbon by Huckerby and Tudor.[25] In the latter case an intense through-space interaction must exist between the amino and the γ-C=O group.

Calvin,[26] in a very interesting paper, emphasizes the interaction of the SH group of the cysteine residue with the carbonyl of the γ-peptide linkage. The hydroxythiazolidine derivative formed could be stabilized by interaction with the deprotonated amino group and elimination of water leading to a pH-dependent dynamic equilibrium and mechanism for ring opening and closure.



(14)

The underlying mechanism for the formation of the strongly reducing $^{\bullet}GS^-$ radical may therefore possibly be the addition of $^{\bullet}OH$ to the cysteine sulphur, or hydrogen abstraction from the CH_2 group in the α-position to the sulphur, leading to the formation of a reducing carbon-centred radical [equation (15)]. This radical is thereafter transferred intramolecularly to a less reducing carbon-centred radical possibly with the structure given by Sjöberg (**1**).



(15)

Acknowledgements

We thank the Natural Science Research Council for financial support. We are grateful for valuable discussions with Drs. J. Lind and G. Merényi.

References

1 L. Sjöberg, T. E. Eriksen, and L. Revesz, *Radiat. Res.*, 1982, **89**, 255.
2 P. Neta and R. W. Fessenden, *J. Phys. Chem.*, 1971, **75**, 738.
3 H. Paul and H. Fischer, *Helv. Chim. Acta*, 1971, **54**, 485.
4 R. Ahmad and D. A. Armstrong, *Can. J. Chem.*, 1984, **62**, 171.
5 B. S. Wolfenden and K. L. Willson, *J. Chem. Soc., Perkin Trans. 2*, 1982, 805.
6 N. H. Anderson and R. O. C. Norman, *J. Chem. Soc. B.*, 1971, 993.
7 M. Simic and X. Hayon, *Int. J. Radiat. Biol.*, 1972, **22**, 507.
8 K. M. Bansal, A. Henglein, and R. M. Sellers, *Ber. Bunsenges. Phys. Chem.*, 1974, **78**, 569.
9 J. C. Sciano, *J. Phys. Chem.*, 1981, **85**, 2851.
10 K. O. Hiller and K. D. Asmus, *J. Phys. Chem.*, 1983, **87**, 3682.
11 K. D. Asmus, M. Göbl, V. O. Hiller, S. Mahling, and J. Mönig, *J. Chem. Soc., Perkin Trans. 2*, 1985, 641.
12 J. Mönig, M. Göbl, and K. D. Asmus, *J. Chem. Soc., Perkin Trans. 2*, 1985, 647.
13 J. Mönig, R. Chapman, and K. D. Asmus, *J. Phys. Chem.*, 1985, **89**, 3139.
14 G. Fransson and T. E. Eriksen, unpublished work.
15 X. Farhataziz and A. B. Ross, NSRDS-NBS, U.S., 1977, **59**, 9.
16 T. E. Eriksen, J. Lind, and T. Reitberger, *Chem. Scr.*, 1976, **10**, 5.
17 D. W. Whillans, *Radiat. Phys. Chem.*, 1977, **10**, 335.
18 M. Z. Barker, R. Badiello, M. Tamba, M. Quintiliani, and G. Gorin, *Int. J. Radiat. Biol.*, 1982, **41**, 595.
19 W. B. Wright, *Acta Crystallogr.*, 1958, **11**, 632.
20 P. R. Rosevear, S. Sellin, B. Mannervik, I. D. Kunz, and A. S. Mildvan, *J. Biol. Chem.*, 1984, **259**, 1436.
21 S. V. Zenin, G. I. Chuprina, and Yu A. Krylova, *Zh. Obschch. Khim.*, 1975, **45**, 1337.
22 S. Fujiwara, G. Formicka-Kozlowska, and H. Kozlowski, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 3131.
23 P. R. Lawrence and C. Thomson, *Theor. Chim. Acta*, 1980, **57**, 25.
24 G. Jung, E. Breitmaier, and W. Voelter, *Eur. J. Biochem.*, 1972, **24**, 438.
25 T. N. Huckerby and A. J. Tudor, *J. Chem. Soc., Perkin Trans. 2*, 1985, 759.
26 M. Calvin in 'Glutathione,' eds. S. Colowick, D. R. Schwarz, A. Lazarow, E. Stadtman, E. Racker, and H. Waelsch, Academic Press, 1954, pp. 1—27.

Received 12*th February* 1987; *Paper* 7/256