Disulfide anion radical equilibria: effects of -NH_3^+, -CO_2^-, -NHC(O)- and -CH_3 groups

Stephen P. Mezyk *† and David A. Armstrong

Department of Chemistry, University of Calgary, Calgary, AB T2N 1N4, Canada

Received (in Cambridge) 10th February 1999, Accepted 13th April 1999

Equilibria between disulfide anion radicals ($RS\therefore\overline{}\ SR$) and thiolate anions and thiyl radicals, namely:

$$RS^{\bullet} + RS^{-} \rightleftharpoons RS\therefore\overline{}\ SR$$

have been studied as a function of pH with alkyl R substituents of different structure and net charge, for the purpose of obtaining thermochemical data. The thermodynamic stability of the $RS\therefore\overline{}\ SR$ species was examined in terms of: (a) the reduction potential for its formation from RSSR, and: (b) the magnitude of the equilibrium constant for its formation from $RS^{\bullet}$ and RS^{-}. It was found that the $RS\therefore\overline{}\ SR$ stability increased when protonated amino groups were present and rose with their proximity to the $S\therefore\overline{}\ S$ group. For each protonated amino group beta to the S atoms E^{o}(RSSR/$RS\therefore\overline{}\ SR$) typically rose by about 0.1 V. In parallel with this the equilibrium constant for formation of $RS\therefore\overline{}\ SR$ from $RS^{\bullet}$ and RS^{-} increased in the doubly protonated systems by a typical factor of ten. This equilibrium constant is strongly depressed by methyl groups on the C atoms adjacent to the sulfur, and is reduced in structures with ionised carboxylate groups beta to the S atom. The magnitude of the latter effect is diminished when the distance between the sulfur centers and the carboxylate groups increased. The changes in $RS\therefore\overline{}\ SR$ stability can be understood in terms of inductive effects and Coulombic interactions. The value of E^{o}(RSSR/$RS\therefore\overline{}\ SR$) = −1.41 V found for glutathione disulfide is an indication of the reduction potential for the cystine residue in proteins. Estimates of the effects of nearby -NH_3^+ groups on the stability of disulfide anions have been made.

Introduction

Thiol and disulfide groups play important roles in a large number of hormones, enzymes and other biologically active proteins.[1,2] The glutathione molecule, the most ubiquitous low molecular weight thiol in cells, is present in roughly millimolar concentrations.[3–5] Glutathione has a number of regulatory functions, and the concentrations of its disulfide (RSSR) and thiol (RSH) forms, which are in equilibrium through reaction (1), are kept at the desired levels by enzyme systems.[1] Disulfide

$$RSSR + 2H^{+} + 2e^{-} = 2RSH \qquad (1)$$

-SS- groups are also present in proteins, where they maintain the tertiary structure, or are involved as essential components of the enzyme active sites.[1,2,6] In a number of these latter cases there has been speculation that the disulfide anion radical, $RS\therefore\overline{}\ SR$, which is an intermediate redox state formed by the addition of a single electron, *viz*. eqn. (2), might be involved in the redox mechanism.

$$RSSR + e^{-} = RS\therefore\overline{}\ SR \qquad (2)$$

Studies of low molecular weight disulfide anion radicals by electron spin resonance and theoretical methods have shown that the extra electron resides in a σ* bond between the two sulfur atoms and the overall bonding is σ(2)σ*(1).[7–11] By convention the three dot symbol in the above formula is used to indicate this.[12] The associated negative charge is indicated here by the superscript minus sign. Disulfide anion radicals are observed when disulfide-containing proteins are reduced by solvated electrons, $CO_2^{\bullet -}$ or other strong reducing agents,[4,10,13–15] and they have been considered as possible intermediates in the intramolecular transfer of an electron from one site to another within a protein.[16]

Disulfide anion radicals are also formed by the reversible association of thiyl radicals and thiolate anions (reaction (3)/(−3)).[4] The pK_as for ionisation of the SH groups of aliphatic

$$RS^{\bullet} + RS^{-} \rightleftharpoons RS\therefore\overline{}\ SR \qquad (3)/(-3)$$

thiols (reaction (4)/(−4)) are typically in the range 8 to 10,[1,2]

$$RSH \rightleftharpoons RS^{-} + H^{+} \qquad (4)/(-4)$$

and formation of $RS\therefore\overline{}\ SR$ by reaction (3) therefore becomes more important at higher pHs. For low molecular weight thiols, like glutathione, cysteine and cysteamine, reactions (3), (−3), (4) and (−4) are fast and generally both of these equilibria are maintained on the microsecond timescale.[17] However, disulfide anion radicals in proteins are stabilised by the tertiary protein structures and do not readily dissociate to thiyl radical and thiolate anion in reaction (−3).[10,18,19]

The complexation of thiyl radicals with thiolate anion in reaction (3) means that $RS\therefore\overline{}\ SR$ also has an important bearing on the removal of free radicals from cells. Damage to biological target molecules, BH, arises from direct action by UV or ionising radiation[4,20] or as a result of attack by oxygen-centered radicals, such as $ROO^{\bullet}$ or $^{\bullet}OH$,[21] *e.g.* reaction (5). The $B^{\bullet}$

$$BH + {}^{\bullet}OH \longrightarrow B^{\bullet} + H_2O \qquad (5)$$

radicals may then react with RSH by H atom transfer (reaction (6)), with some $RS\therefore\overline{}\ SR$ being formed subsequently *via*

$$B^{\bullet} + RSH \longrightarrow BH + RS^{\bullet} \qquad (6)$$

† Present address: Reactor Chemistry Branch, Atomic Energy of Canada Ltd., Chalk River, ON K0J 1J0, Canada.

reaction (3).[4,5,22] The most promising processes for removal of $RS^{\bullet}$ appear to be the direct reaction of $RS^{\bullet}$ with cellular antioxidants, such as ascorbate (Asc^{-}), *viz*. reaction (7), or the

$$RS^{\bullet} + Asc^{-} + H^{+} \longrightarrow RSH + Asc^{\bullet} \qquad (7)$$

reaction of $RS\therefore SR$ with oxygen, reaction (8).[23,24] The $O_2^{\bullet -}$ anion is then removed by superoxide dismutase.[22]

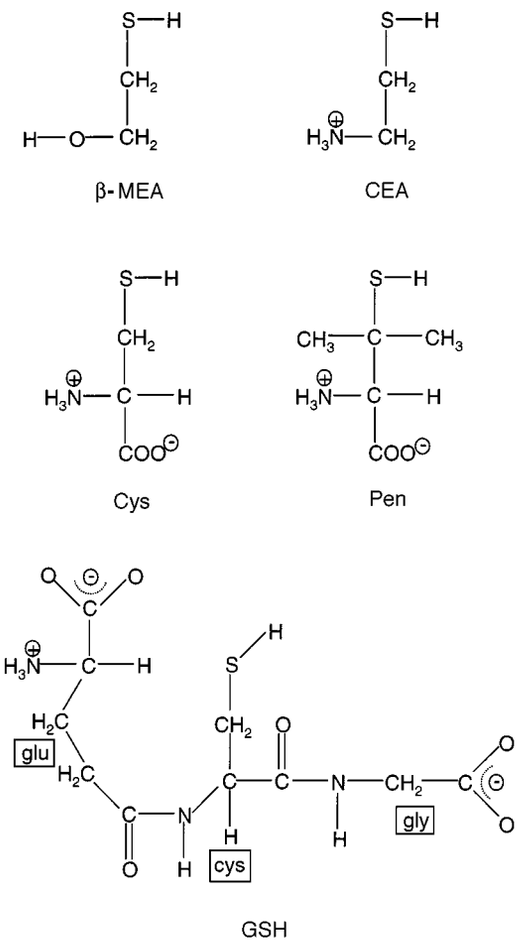
$$RS\therefore SR + O_2 \longrightarrow RSSR + O_2^{\bullet -} \qquad (8)$$

Recently one of us has performed systematic studies of the effect of pH on equilibria between thiyl and disulfide anion radicals in the presence of free thiols.[25–29] The thiols used contained varying numbers of positive $-NH_3^+$ groups, and for each sulfanyl structure the equilibrium constants were determined over the pH range 6 to 13. The results demonstrated a clear difference between β-mercaptoethanol (β-MEA), which has no amino groups, and the amino-containing thiols. For the latter compounds the equilibrium constant rose from a low value below pH 7 and passed through a sharp maximum between pH 8 and 9. It then fell, reaching a level which remained constant above pH ~10. For β-MEA it simply rose from the pH 7 value to the plateau with no pronounced maximum. This difference in behaviour suggests that protonated $-NH_3^+$ groups are present on the disulfide anions in the pH region 7 to 9 and stabilise them against reaction (−3). Another feature was that for different amino-thiol structures the equilibrium constants at a given pH were found to vary by a factor of ten or more.

This paper is concerned with a detailed analysis of these results and a comparison of the stabilities with respect to reaction (−3) of disulfide anions of different structure. Values of K_3 for specific charge forms of thiol, thiyl radical and disulfide anion are found. Also the pK_as of the ionisable $-NH_3^+$ groups in the $RS\therefore SR$ anions and the values of E°(RSSR/$RS\therefore SR$), the reduction potential for the half reaction (2) above, have been calculated with two, one and zero amino groups of the disulfide anions protonated. Although these anion radicals have been studied for many years, this appears to be the first systematic analysis of the effects of neighbouring charged groups and other structural factors on the stability of the $S\therefore S$ group. The work is important for an understanding of the possible role of $S\therefore S$ groups in electron transfer reactions in proteins and their involvement in the active centers of redox enzymes. It may also be relevant to mechanisms for the removal of radicals from cells, since in principle $RS^{\bullet}$ radicals may undergo reaction (3) with sulfanyl groups of enzymes and hormones, as well as GSH.

Methods

The thiols used were β-mercaptoethanol (β-MEA), cysteamine (CEA), cysteine (Cys), penicillamine (Pen) and the tripeptide glutathione (GSH). The structures of these compounds are shown in Scheme 1. Since the carboxy groups of Cys, Pen and GSH are fully ionised in the pH 5 to 13 region,[30,31] one need only be concerned with ionisation equilibria for the sulfanyl and amino groups. The uncomplexed thiols may be present both as neutral and thiolate anion forms. Also, for the thiols other than β-MEA, the sulfanyl (-SH) and thiolate anion ($-S^{-}$) forms, the thiyl radicals and the disulfide anion radicals all have ionisable $-NH_3^+$ groups. The acid/base forms of the thiyl radicals, the uncomplexed thiols and the disulfide anions, are shown in Fig. 1. The formulae show only the sulfanyl, amino and $S\therefore S$ groups. For convenience $\langle MS^{\bullet}\rangle$, $\langle MS^{-}\rangle$ and $\langle MS\therefore SM\rangle$ are used to represent thiyl radical, thiolate anion and disulfide anion radical species, respectively, and the number of protonated amino groups present is indicated where needed by a subscript 0, 1 or 2. A subscript T is used to refer to the sum of all acid/base forms.



Scheme 1

Equilibria

The total concentrations of all acid/base forms of the radical ($[\langle MS^{\bullet}\rangle_T]$), thiol ($[\langle MS^{-}\rangle_T]$) and disulfide anion radical ($[\langle MS\therefore SM\rangle_T]$) at a specific pH are given by the relations (9)–(11). The experimental equilibrium constants for thiyl radical–

$$[\langle MS^{\bullet}\rangle_T] = [\langle MS^{\bullet}\rangle_1] + [\langle MS^{\bullet}\rangle_0] \qquad (9)$$

$$[\langle MS^{-}\rangle_T] = [\langle MSH\rangle_1] + [\langle MSH\rangle_0] + [\langle MS^{-}\rangle_1] + [\langle MS^{-}\rangle_0] \qquad (10)$$

$$[\langle MS\therefore SM\rangle_T] = [\langle MS\therefore SM\rangle_2] + [\langle MS\therefore SM\rangle_1] + [\langle MS\therefore SM\rangle_0] \qquad (11)$$

disulfide anion formation in references 25–29 were reported in terms of these total concentrations. The equilibrium constants (K_T s) are therefore complex "overall" constants defined by expression (12).

$$K_T = \frac{[\langle MS\therefore SM\rangle_T]}{[\langle MS^{\bullet}\rangle_T][\langle MS^{-}\rangle_T]} \qquad (12)$$

The major objective of this study was to analyse the pH dependences of these equilibrium constants so that the pK_as of the two $-NH_3^+$ groups on the disulfide anions of the amino thiols could be determined and the relative stabilities of the different protonated forms estimated. The pK_as of the thiols can be obtained from literature data. Thus the pH dependence of K_T must be fitted by a function which incorporates these data as input and fixes the pK_as of the $-NH_3^+$ groups of the disulfide anion radicals. That function is described next.

Fitting function

Since the forms of the thiyl radicals, thiols and the disulfide

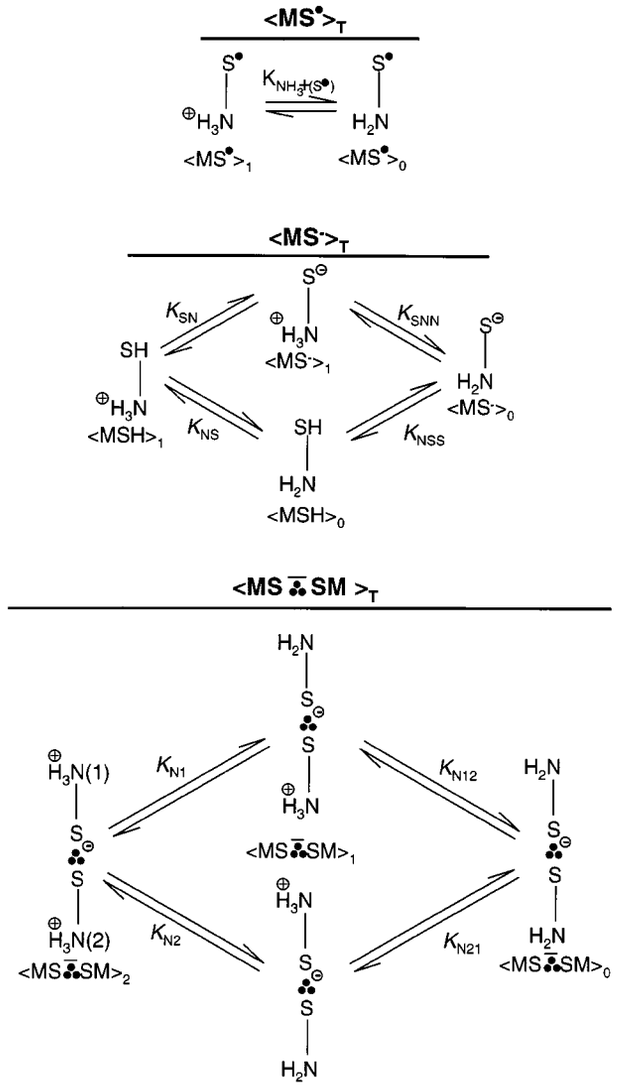


Fig. 1 The acid/base forms of the thiyl radicals (⟨MS•⟩), the uncomplexed thiols (⟨MS⁻⟩) and the disulfide anions ⟨MS∴SM⟩. Note that for MS∴SM $K_{N1} = K_{N2}$ and $K_{N21} = K_{N12}$.

anions, which contain no ionisable protons (⟨MS•⟩$_0$, ⟨MS⁻⟩$_0$ and ⟨MS∴SM⟩$_0$, respectively) will be the only ones present at high pH, it is convenient to use the equilibrium (3p0)/(−3p0) as a

$$\langle MS^{\bullet}\rangle_0 + \langle MS^{-}\rangle_0 \rightleftharpoons \langle MS^{\therefore}SM\rangle_0 \qquad (3p0)/(-3p0)$$

basis for further development. The equilibrium constant K_{p0} is defined by expression (13), which can be rewritten as eqn. (14),

$$K_{p0} = \frac{[\langle MS^{\therefore}SM\rangle_0]}{[\langle MS^{\bullet}\rangle_0][\langle MS^{-}\rangle_0]} \qquad (13)$$

$$K_{p0} = \frac{[\langle MS^{\therefore}SM\rangle_T]}{[\langle MS^{\bullet}\rangle_T][\langle MS^{-}\rangle_T]} \times \frac{f(ss)_0}{f(s^{\bullet})_0 f(s^{-})_0} \qquad (14)$$

where $f(ss)_0$, $f(s^{\bullet})_0$ and $f(s^{-})_0$ are the fractions of ⟨MS∴SM⟩$_T$, ⟨MS•⟩$_T$ and ⟨MS⁻⟩$_T$ present as ⟨MS∴SM⟩$_0$, ⟨MS•⟩$_0$ and ⟨MS⁻⟩$_0$, respectively, at the pH of measurement. From (12) and (14) one obtains (15).

$$K_T = K_{p0} \times \frac{f(s^{\bullet})_0 f(s^{-})_0}{f(ss)_0} \qquad (15)$$

By using the standard treatment of equilibria for polyprotic acids,[32] the values of the *f* parameters can be written in terms of the ionisation constants for the specific groups as defined in Fig. 1. These are normally referred to as microscopic ionisation constants (see further below). The *f*s are given in eqns. (16)–(18).

$$f(s^{\bullet})_0 = \left\{\frac{K_{NH_3^+(S^{\bullet})}}{K_{NH_3^+(S^{\bullet})} + [H^+]}\right\} \qquad (16)$$

$$f(s^{-})_0 = \left\{\frac{K_{SN}K_{SNN}}{[H^+]^2 + K_{SN}[H^+] + K_{NS}[H^+] + K_{SN}K_{SNN}}\right\} \qquad (17)$$

$$f(ss)_0 = \left\{\frac{K_{N1}K_{N12}}{[H^+]^2 + K_{N1}[H^+] + K_{N2}[H^+] + K_{N1}K_{N12}}\right\} \qquad (18)$$

There is no experimental information on $K_{NH_3^+(S^{\bullet})}$ for any of the compounds used here, and therefore it was also determined from the fitting process. The ionisation constants K_{SN}, K_{NS}, K_{SNN} and K_{NSS} for the thiols used here have been determined by spectroscopic or NMR methods. Since K_{SN} and K_{NS} are not widely separated, the macroscopic ionisation constants K_S' and K_S'', which are measured by titrimetric methods, involve participation from both the SH and -NH_3^+ groups. The micro- and macroscopic constants are related by eqns. (19) and (20),[32] and

$$K' = K_{SN} + K_{NS} \qquad (19)$$

$$K_S'K_S'' = K_{SN}K_{SNN} = K_{NS}K_{NSS} \qquad (20)$$

it is convenient to rewrite $f(s^{-})_0$ in the simpler form of eqn. (21).

$$f(s^{-})_0 = \left\{\frac{K_s'K_s''}{[H^+]^2 + K_s'[H^+] + K_s'K_s''}\right\} \qquad (21)$$

In a similar way there will be two macroscopic constants K_{SS}' and K_{SS}'' for the ionisation of the -NH_3^+ groups in the disulfide anions, and the relations to the microscopic constants are given by eqns. (19a) and (20a). However, in this case $K_{N1} = K_{N2}$ and

$$K_{SS}' = K_{N1} + K_{N2} \qquad (19a)$$

$$K_{SS}'K_{SS}'' = K_{N2}K_{N21} = K_{N1}K_{N12} \qquad (20a)$$

$K_{N12} = K_{N21}$. Thus one has eqn. (22). For β-MEA, where there

$$f(ss)_0 = \left\{\frac{K_{ss}'K_{ss}''}{[H^+]^2 + K_{ss}'[H^+] + K_{ss}'K_{ss}''}\right\} \qquad (22)$$

are no -NH_3^+ groups, $f(s^{\bullet})_0$ and $f(ss)_0$ are unity, and $f(s^{-})_0$ is given by eqn. (23).

$$f(s^{-})_0 = \left\{\frac{K_s'}{[H^+] + K_s'}\right\} \qquad (23)$$

Data fitting

Expression (15), with the simplified forms of $f(ss)_0$, $f(s^{\bullet})_0$ and $f(s^{-})_0$, was used in the Levenberg–Marquardt nonlinear least-squares fitting module within the Origin computer program (MicroCal Software, Inc., Northampton, MA) to fit the pH dependence of K_T for each compound. The microscopic and macroscopic ionisation constants of the thiols in Table 1,[31,33,34] which are self consistent in terms of eqns. (19) and (20), were used as input, and the values of K_{p0}, $K_{NH_3^+(S^{\bullet})}$, K_{SS}' and K_{SS}'' were varied to minimize Chi-squared.

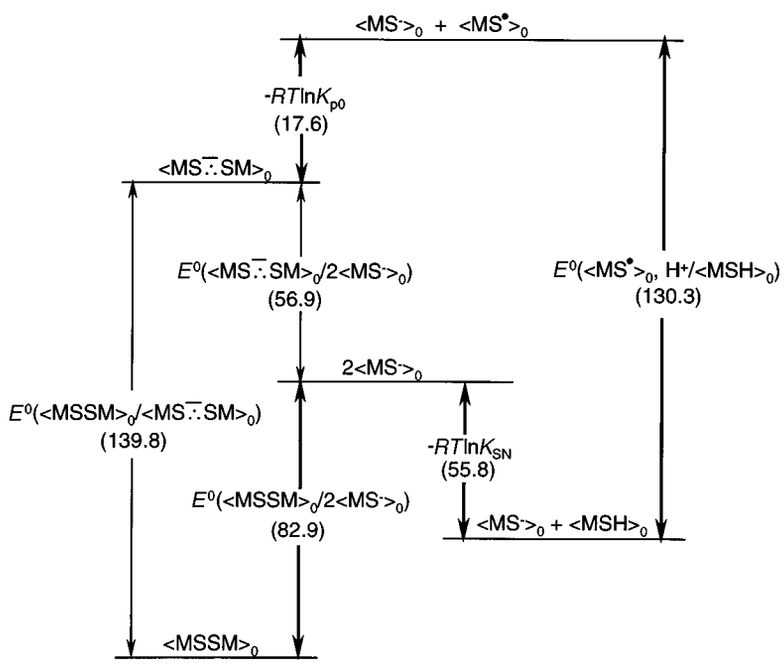
Reduction potentials

Deprotonated disulfides. The values of K_{p0} determined here were used along with literature data for K_{NSS}, and the reduction

Table 1 Properties of thiols (⟨MSH⟩) and thiyl radicals (⟨MS•⟩)

		$pK_{S'}$	$pK_{S''}$	pK_{SN}	pK_{NS}	pK_{SNN}
β-MEA						
	⟨MSH⟩	**9.53**[a]	—	—	—	
CEA						
	⟨MSH⟩	8.30[b]	10.75[b]	8.35[c]	9.27[d]	10.70[d]
	⟨MS•⟩	**9.11 ± 0.12**	—	—	—	—
Cys						
	⟨MSH⟩	8.36[e]	10.52[e]	8.52[e]	8.86[e]	10.36[e]
	⟨MS•⟩	**8.26 ± 0.04**	—	—	—	—
Pen						
	⟨MSH⟩	7.90[f]	10.46[f]	8.02[f]	8.50[f]	10.33[f]
	⟨MS•⟩	**8.12 ± 0.09**	—	—	—	—
GSH						
	⟨MSH⟩	8.75[b]	9.65[b]	8.93[g]	9.13[g]	9.47[g]
	⟨MS•⟩	**9.08 ± 0.02**	—	—	—	—

[a] Fitted value in this study. Results of this study in bold face. [b] From reference 31. [c] From reference 1. [d] Calculated from $pK_{S'}$, $pK_{S''}$ and pK_{SN}. [e] Based on the averages of the microscopic constants determined by resonance raman and UV spectroscopy in reference 1 and $pK_{S'}$ and $pK_{S''}$ calculated from them. [f] From reference 33. [g] pK_{SN} and pK_{NS} from reference 34. The pK_{SNN} value in reference 34 has a much larger error than the other pK_as. The present value was calculated from pK_{NS}, $pK_{S'}$ and $pK_{S''}$.



Scheme 2

potentials $E^{\circ}(\langle MSSM\rangle_0/2\langle MS^-\rangle)$ and $E^{\circ}(\langle MS^{\bullet}\rangle_0,H^+/\langle MSH\rangle_0)$ to calculate the reduction potentials for the *deprotonated* disulfides in the half reactions (2p0) and (24p0).

$$\langle MSSM\rangle_0 + e^- = \langle MS\therefore SM\rangle_0,\ E^{\circ}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0) \quad (2p0)$$

$$\langle MS\therefore SM\rangle_0 + e^- = 2\langle MS^-\rangle_0,\ E^{\circ}(\langle MS\therefore SM\rangle_0/2\langle MS^-\rangle_0) \quad (24p0)$$

The relations between the known parameters and the ones calculated here are shown in Scheme 2. The known parameters are distinguished by the heavier arrows. The values of $E^{\circ}(\langle MSSM\rangle_0/2\langle MS^-\rangle_0)$ were obtained from the $E^{\circ}(\langle MSSM\rangle_2, 2H^+/2\langle MSH\rangle_1)$ results in reference 35 and the literature pK_as of the thiols and disulfides, which are given in Tables 1 and 2, respectively. $E^{\circ}(\langle MS^{\bullet}\rangle_0,H^+/\langle MSH\rangle_0)$ was taken to be 1.35 V for all of the thiols used here.[36] The reason for that is that there is good evidence that $E^{\circ}(RS^{\bullet},H^+/RSH)$ for alkyl thiols is relatively insensitive to the structure of the alkyl R group.[37] This is also true for $E^{\circ}(\langle MSSM\rangle_2,2H^+/2\langle MSH\rangle_1)$, which differs by only 0.02 V over the series of thiols studied here.[35] One should note that the single literature value of $E^{\circ}(\langle MSSM\rangle_2,2H^+/2\langle MSH\rangle_1)$ previously used for MEA[37] was ~0.17 V larger than the experimental values subsequently reported in reference 35 and used here and in reference 36. The values of $E^{\circ}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$ calculated here and in reference 36 are considered to be more correct.

Protonated disulfides. The E° values for the half reactions (2p1) and (24p1) of the *monoprotonated* forms of the disulfides were calculated from $E^{\circ}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$, $E^{\circ}(\langle MS\therefore SM\rangle_0/2\langle MS^-\rangle_0)$, K_{SS}', K_{SS}'' and the pK_as of the parent disulfides. Since the latter each have two ionisable $-NH_3^+$ groups, their ionisation patterns are analogous to those of the disulfide anions in Fig. 1. The self consistent micro- and macroscopic pK_as for them were obtained from the literature and are listed in Table 2.[30,38,39] In a similar way, E° values for half reactions (2p2) and (24p2) of the *diprotonated* forms were obtained.

$$\langle MSSM\rangle_1 + e^- = \langle MS\therefore SM\rangle_1;\ E^{\circ}(\langle MSSM\rangle_1/\langle MS\therefore SM\rangle_1) \quad (2p1)$$

Table 2 Properties of ⟨MSSM⟩ disulfides and ⟨MS∴ SM⟩ disulfide anion radicals[a]

		p$K_{SS'}$	p$K_{SS''}$	K_{p0}	K_{p1}	K_{p2}
β-MEA						
	⟨MS∴ SM⟩	—	—	**1440 ± 40**	—	—
CEA						
	⟨MS∴ SM⟩	**10.11 ± 0.22**	**10.43 ± 0.17**	**1234 ± 108**	**663**	**6600**
	⟨MSSM⟩	8.82[b]	9.58[b]	—	—	—
Cys						
	⟨MS∴ SM⟩	**9.41 ± 0.08**	**10.52 ± 0.07**	**438 ± 32**	**317**	**8900**
	⟨MSSM⟩	8.03[c]	8.80[c]	—	—	—
Pen						
	⟨MS∴ SM⟩	**9.25 ± 0.14**	**10.50 ± 0.13**	**33.2 ± 6**	**25**	**660**
	⟨MSSM⟩	7.60[d]	8.80[d]	—	—	—
GSH						
	⟨MS∴ SM⟩	**9.12 ± 0.07**	**9.98 ± 0.05**	**1987 ± 61**	**3200**	**7050**
	⟨MSSM⟩	8.57[e]	9.54[e]	—	—	—

[a] Results of this study in bold face. [b] From reference 38. [c] From reference 39. [d] From reference 33(*a*). [e] From reference 30.

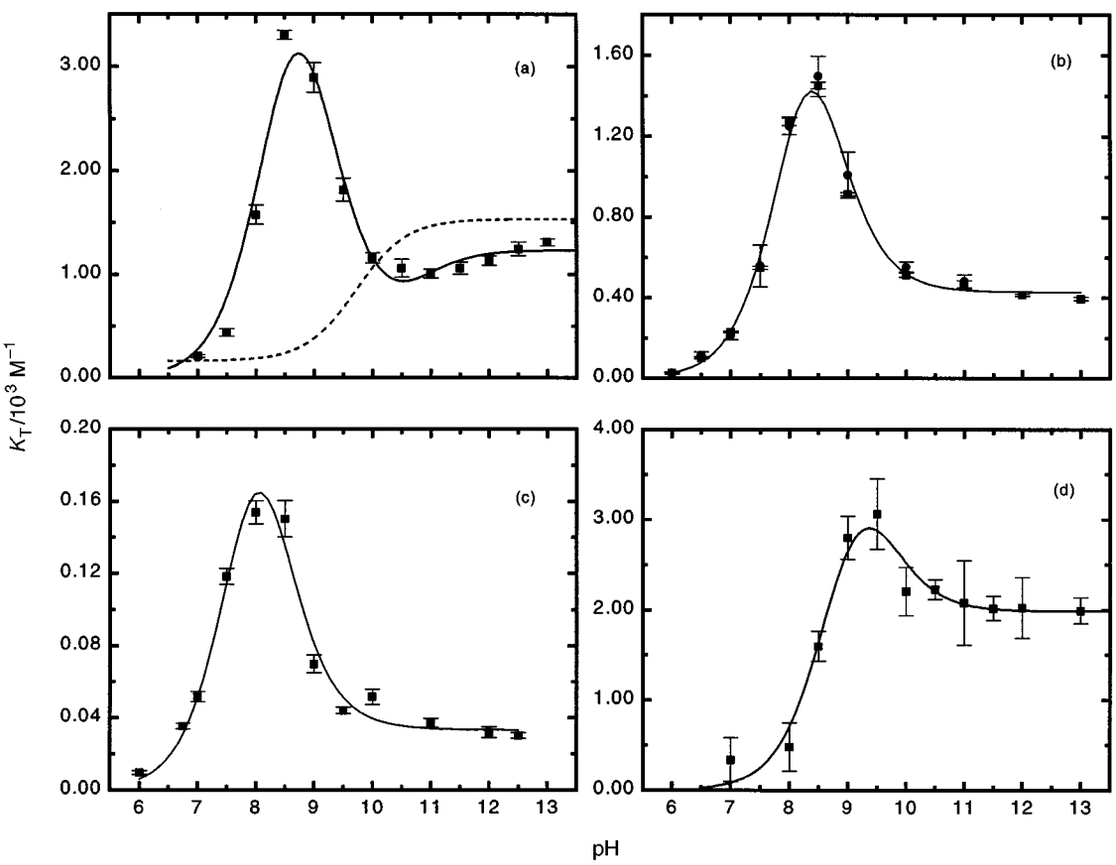


Fig. 2 Experimental data for the equilibrium constants K_T as a function of pH for: (a) CEA (points), MEA (dashed line) from reference 27; (b) Cys from reference 29; (c) Pen; (d) GSH from reference 28. Squares are integrated yield measurements, circles kinetic measurements. Solid lines are fits to eqn. (15).

$$\langle MS\therefore SM\rangle_1 + e^- = \langle MS^-\rangle_0 + \langle MS^1\rangle_1;\ E^o(\langle MS\therefore SM\rangle_1/\langle MS^-\rangle_0,\langle MS^-\rangle_1) \quad (24p1)$$

$$\langle MSSM\rangle_2 + e^- = \langle MS\therefore SM\rangle_2;\ E^o(\langle MSSM\rangle_2/\langle MS\therefore SM\rangle_2) \quad (2p2)$$

$$\langle MS\therefore SM\rangle_2 + e^- = 2\langle MS^-\rangle_1;\ E^o(\langle MS\therefore SM\rangle_2/2\langle MS^-\rangle_1) \quad (24p2)$$

Results

The experimental values of K_T at different pHs are shown by the points in Fig. 2a,b,c and d for CEA, Cys, Pen and GSH, respectively. They have been taken from references 25–29, and the values are correct to ±10 percent. The lines through the points are the computed fits, based on simultaneously varying the unknown parameters in the *f* values of eqn. (15) to achieve the best agreement with the experimental data. For β-MEA the experimental points from reference 27 have not been presented, but the best fit line is shown by the dashed curve in Fig. 2a.

The fitting procedure gives K_{p0}, K_{SS}' and K_{SS}'' for the amino-containing disulfide anion radicals and $K_{NH_3^+(S^\cdot)}$ for the thiyl radicals. Values of $K_{NH_3^+(S^\cdot)}$ are given in Table 1 under pK_S', since the ⟨MS$^\cdot$⟩$_1$ species has only one ionisable proton. Those for K_{p0}, K_{SS}' and K_{SS}'' have been presented in Table 2. The quoted errors are one standard deviation as obtained from the fitting process. In general the errors in the pK_as are ±0.15 or less. The errors for K_{SS}' and K_{SS}'' of CEA, where the two pK_as are very close, are somewhat larger (±~0.2, see Table 2). Except for Pen, where K_{p0} is relatively small, the errors in this

Table 3 Reduction potentials (V) of ⟨MSSM⟩ disulfides and ⟨MS∴ SM⟩ disulfide anion radicals

	E^{o}(⟨MSSM⟩/⟨MS∴ SM⟩)			E^{o}(⟨MS∴ SM⟩/⟨2MS$^-$⟩)		
No. of NH_3^+	2	1	0	2	1	0
β-MEA	—	—	−1.40	—	—	0.60
CEA	−1.32	−1.38	−1.45	0.64	0.61	0.59
Cys	−1.30	−1.38	−1.50	0.65	0.61	0.60
Pen	−1.34	−1.44	−1.54	0.72	0.68	0.68
GSH	−1.35	−1.38	−1.41	0.60	0.59	0.61

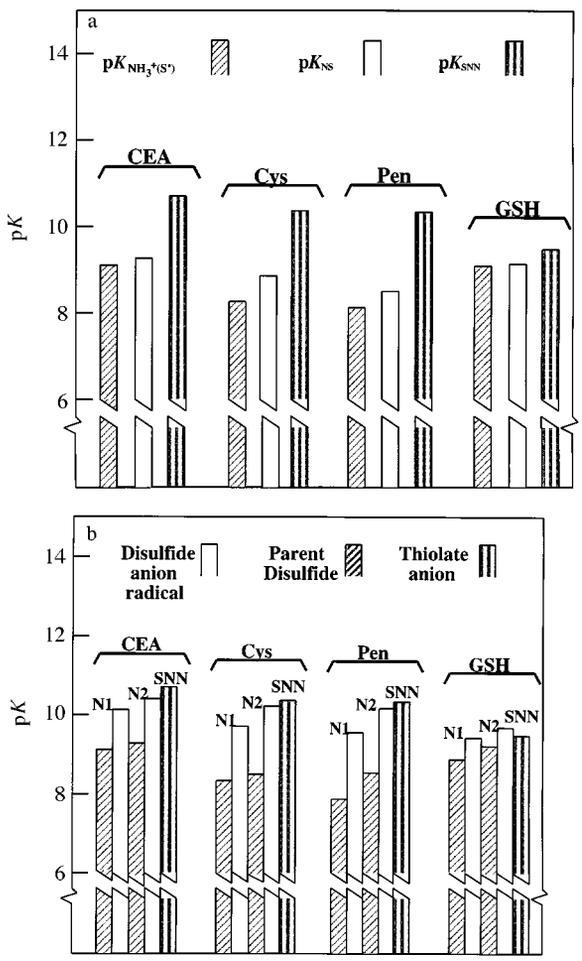


Fig. 3 Comparisons of pK_a values. (a) $pK_{NH_3^+(S^\bullet)}$ ⟨MS$^\bullet$⟩, pK_{NS} for ⟨MSH⟩ and pK_{SNN} for ⟨MS$^-$⟩; (b) pK_{N1} (N1) and pK_{N2} (N2) for parent disulfides and disulfide anion radicals and pK_{SNN} for ⟨MS$^-$⟩.

parameter are less than 10 percent. It should be noted here that attempts to fit the data with a single pK_a for the disulfide anions lead to very large errors and were totally unsuccessful. Likewise fixing the pK_a of the -NH_3^+ in the MS$^\bullet$ radical ($K_{NH_3^+(S^\bullet)}$) to be the same as in MSH (*i.e.* K_{NS}) caused very poor fits.

The values of $K_{NH_3^+(S^\bullet)}$ have been compared with pK_{NS} and pK_{SNN}, the pK_as for the parent ⟨MSH⟩$_1$ and ⟨MS$^-$⟩$_1$ non-radical species, respectively, in Fig. 3a. For the disulfide anion radicals the values K_{N1} and K_{N12} were derived from K_{SS}' and K_{SS}'' by using expressions (19a) and (20a) and used to calculate pK_{N1} and pK_{N12}. These are compared with pK_{N1} and pK_{N12} for the parent disulfides and pK_{SNN} for the parent thiolate ion in Fig. 3b.

Equilibrium constants have also been presented in Table 2 for the formation of disulfide anions with one and two amino groups protonated according to the equilibria (3p1)/(−3p1) and (3p2)/(−3p2). The equilibrium constants for (3p1)/(−3p1) and (3p2)/(−3p2) are written as K_{p1} and K_{p2}, respectively. It may be noted that the alternative equilibrium to (3p1)/(−3p1) for the dissociation of ⟨MS∴ SM⟩$_1$ into ⟨MS$^\bullet$⟩$_1$ and ⟨MS$^-$⟩$_0$ was not included, since ⟨MS$^\bullet$⟩$_1$ with a neutral -S$^\bullet$ should deprotonate at a lower pH than ⟨MS$^-$⟩$_1$ with a negatively charged -S$^-$. It is also reasonable, because $pK_{NH_3^+(S^\bullet)}$ was significantly less than pK_{SNN} (see Table 1) for all amino thiols. K_{p1} is given by the relation $K_{p1} = K_{p0} \times (K_{SNN}/K_{N12})$, and $K_{p2} = K_{p0} \times (K_{SNN}/K_{N12})$-$(K_{NH_3^+(S^\bullet)}/K_{N1})$. Because they are calculated from the pK_as, the K_{p1} and K_{p2} values are subject to considerably greater uncertainty than the K_{p0}s.

$$\langle MS^\bullet\rangle_0 + \langle MS^-\rangle_1 \Leftrightarrow \langle MS\therefore SM\rangle_1 \qquad (3p1)/(-3p1)$$

$$\langle MS^\bullet\rangle_1 + \langle MS^-\rangle_1 \Leftrightarrow \langle MS\therefore SM\rangle_2 \qquad (3p2)/(-3p2)$$

The reduction potentials are given in Table 3. Scheme 2 illustrates the relation between K_{p0}, K_{SN} and the reduction potentials. The numbers in parenthesis for each step are the ΔG° changes in kJ mol^{-1} for the particular case of CEA. It may be noted that, despite the large variations in the K_{p0} values in Table 2, the free energy change in reaction (3p0) varies only from 8.7 kJ mol^{-1} for Pen to 18.8 kJ mol^{-1} for GSH.

Discussion

The difference between the pH dependence of K_T for β-MEA and the amino-containing compounds is quite obvious from a comparison of the dashed curve in Fig. 2a with the data points for CEA in the same figure and for the other amino thiols in Figs. 2b–d. For β-MEA the initial rise in K_T, which occurs near pH 7 for the amino thiols, is delayed to pH 9, and the sharp maximum in the region of pH 8.5 to 9.0, to which reference was made in the Introduction, is absent. Also notable is the variation in the values of K_{p0}, K_{p1} and K_{p2} in Table 2 between the different amino thiols. Clearly substitutions in the aliphatic chain can have a marked effect on the ⟨MS∴ SM⟩ stability, the most obvious being that produced by -NH_3^+ groups. At the same time one can see from Tables 1 and 2 that the -S$^\bullet$ and S∴ S groups have a significant effect on the pK_as of the -NH_3^+ groups. Group substitutions produce changes in molecular properties by inductive and charge effects, and also in some cases by steric effects.[12,40] The changes in pK_as of aliphatic amines and carboxylic acids caused by substitutions have been well documented [30,41] and it is convenient to begin by examining the effects of the -S$^\bullet$ and S∴ S groups on the -NH_3^+ pK_as in the ⟨MS$^\bullet$⟩ and ⟨MS∴ SM⟩ species.

pK_as

$K_{NH_3^+(S^\bullet)}$. Perrin *et al.*[41] have given linear free energy relations for predicting pK_a changes from Hammett σ^* values of substituents as a function of the number of carbon atoms between them and the -NH_3^+ group. Electron withdrawing groups with large positive σ^* values are base weakening (reduce the pK_a) and donating groups with negative σ^* values have the opposite effect. The -SH group is in the former category. This is readily seen from the fact that the -NH_3^+ pK_a for CEA, pK_{NS} in Table 1 (9.27), is much less than that of unsubstituted $CH_3CH_2NH_3^+$ (pK_a = 10.7[31]). The values in Table 1 for Cys and Pen reflect the additional substitutions of carboxylate at the β position and, for Pen, CH_3 at the α position. One would expect -S$^\bullet$ to have a stronger base weakening effect than -SH, since the

former is more electron deficient. Also -S$^{\bullet}$ has been observed[42] to depress the pK_a of the hydroxy group in *p*-HOC$_6$H$_4$S$^{\bullet}$ to 4.85 from 10.0 in phenol.‡ The -S$^-$ group on the other hand will act to increase the base strength, due to its negative charge and greater electron donating ability than that of SH.[42] Reference to Fig. 3a, shows that the -NH_3^+ pK_as of $\langle MS^{\bullet}\rangle_1$, $\langle MSH\rangle_1$ and $\langle MS^-\rangle_1$ ($pK_{NH_3^+(S^{\bullet})}$, pK_{NS} and pK_{SNN}, respectively) increase in that order, in accord with this prediction. Qualitatively similar behaviour is seen for GSH, but the differences here are minimal. This can be attributed to the increase in the distance between the -NH_3^+ group and the S atom from two carbon atoms in CEA, Cys and Pen to seven C/N linking atoms in GSH (Scheme 1).

Disulfide pK_as. The values of pK_{N1} and pK_{N12} for the anion radicals and the parent disulfides are compared in Fig. 3b. The presence of the extra electron and negative charge in the S$\therefore$ S group increases the pK_as significantly for CEA, Cys and Pen, as would be expected from inductive and charge effects. The upper limit for pK_{N12} should be given by pK_{SNN}, the -NH_3^+ pK_a of the uncomplexed thiolate anion with a full negative charge on one S atom. pK_{SNN} is also given in Fig. 3b, and it can be seen that the values of pK_{N12} do approach it. Similar trends are seen for GSH, but, due to the greater separation of the -NH_3^+ s from the sulfur atoms, the effect is again smaller.

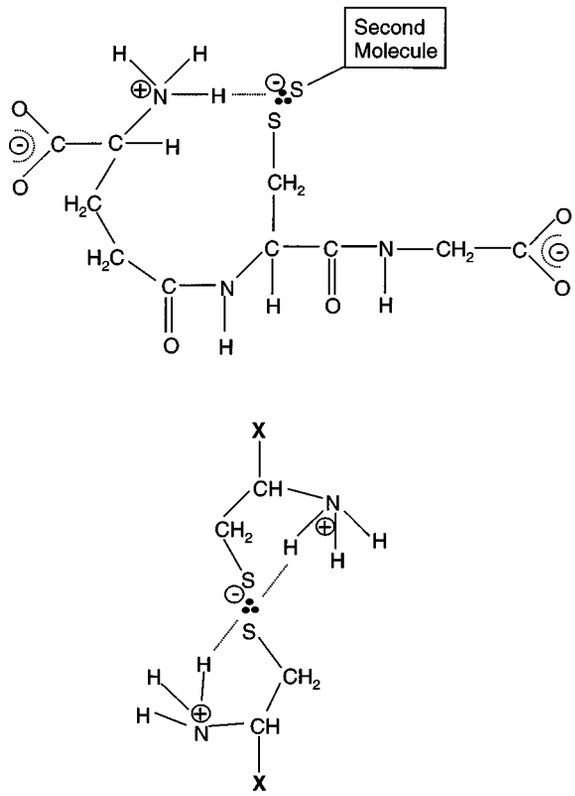
Equilibrium constants, K_{p0}, K_{p1} and K_{p2}

Two factors will contribute to the values of the free energy changes, which determine the equilibrium constants K_{p0}, K_{p1} and K_{p2}. These are: (a) the strengths of the -S$\therefore$ S- bonds, and (b) differences in the solvation energies of the reactants and products. Here the assumption is made that for each of the three equilibria the latter quantity is similar for the different thiols. Attention is first confined to the differences in K_{p0}, and they are interpreted in terms of the strengths of the -S$\therefore$ S- bonds. Asmus[12] has discussed the effects of changes in aliphatic R groups on σ(2)σ*(1) bonding. For RS$\therefore$ SR systems inductive effects are more important than steric. Also the observation that K_{p0} for HS$\therefore$ SH is some ten times larger than the value for β-MEA[43] disulfide anion strongly supports the concept that the -S$\therefore$ S- bond strength decreases with an increase in the electron donating ability of the R group.

Of the systems used here β-MEA and CEA are the simplest. At high pH the disulfide anion radicals of both have total charges of −1 and differ only in the change of the -OH group on the beta C atom into an -NH_2 group. One would therefore expect only a minor difference in inductive effects, and the similar values of K_{p0} in Table 2, 1440 and 1228 for β-MEA and CEA respectively, bear this out. For Cys the R group differs from that of CEA in having a negative carboxylate on the beta C atom (X = -CO_2^- in Scheme 3). These substituents will change the electron densities in the R groups and the negative charges will be repelled by each other and by the negative -S$\therefore$ S- group. The fact that the value of K_{p0} for Cys is almost a factor of three lower than K_{p0} for CEA shows that this significantly reduces the radical anion stability. Pen differs from Cys only in the presence of the methyl groups alpha to the thiol, and this has a major effect on K_{p0}, which is smaller by a factor of ten than for Cys. That effect must be attributed to weakening of the -S$\therefore$ S- σ(2)σ*(1) bond by the increase in the electron density in the σ* antibonding orbital due to donation from the methyl groups.[12]

The roughly tenfold increase in K_{p2} over K_{p0} in Table 2 for the disulfide anions of CEA, Cys, and Pen is very striking. While the absolute values of K_{p2} have some uncertainty, the large increases explain the origin of the sharp maxima in the pH dependences of the experimental K_T values. The presence of the two -NH_3^+ groups in the $\langle MS\therefore SM\rangle_2$ forms must stabilise the

‡ Comparison with the ΔpK_as seen in phenoxyl systems with Br, Cl, CN and NO_2 suggests a σ^* value of about 5.



Scheme 3

-S$\therefore$ S- groups by Coulombic interactions (see Scheme 3) and strengthen the σ(2)σ*(1) bond by inductive effects. In contrast, reference to Table 2 shows that the values of K_{p1} are smaller than those of K_{p0}. In the case of CEA, where this decrease is largest, it is reflected in the slight minimum in the experimental K_T data at pH 10.5 in Fig. 2a. Since it is unreasonable to expect destabilisation by a single -NH_3^+, it appears that for the distribution of charges in equilibrium (3p1)/(−3p1) solvation must favour the dissociation products.

As shown in Schemes 1 and 3, the amino and carboxy groups of GSH are much further from the S atom than in the other thiols. Thus, the interactions with the -S$\therefore$ S- group should be smaller and they are expected to have less effect. More important in this tripeptide are the conversions of the Cys amino and carboxylate groups into peptide linkages (see Scheme 1). As seen from Table 2 the overall effect is to increase K_{p0} by a factor of 4 to 5 over the value for Cys. K_{p0} for GSH is in fact the largest seen here, being even greater than the value for CEA. This is despite the fact that the $\langle MS\therefore SM\rangle_0$ form of GSH bears a net charge of −5, and one might have expected this to facilitate dissociation. To explain that feature it must be realised that the electron donating ability of the cysteinyl amino group in the glutamyl–cystinyl linkage of the tripeptide will be greatly reduced from that of a free -NH_2. Likewise conversion of the cysteinyl carboxy group into the -C(O)-NH- linkage between the Cys and Gly residues will increase the electron withdrawing ability above that of -CO_2^-. Thus the R group of GSH can be expected to have an overall greater electron withdrawing effect than either of the other R groups present here. At the same time the Coulombic interaction between the negative -CO_2^- groups on the β-carbon atoms and the -S$\therefore$ S- groups is removed. Obviously these two factors strongly enhance the σ(2)σ*(1) bond strength and K_{p0} for this $\langle MS\therefore SM\rangle_0$.

Unlike the other amino thiols, for GSH K_{p1} is larger than K_{p0}, and there is then a further increase for K_{p2}. In view of the much larger distances between the -S$\therefore$ S- and amino groups on the carbon skeleton of this compound it seems likely that in this case the increases are mainly due to through space interactions, as depicted in Scheme 3. The increased flexibility of the larger

aliphatic groups will make formation of these more facile than for CEA, Cys and Pen.

Reduction potentials

The $E^{o}(\langle MSSM\rangle_x/\langle MS\therefore SM\rangle_x)$ values in Table 3 are negative, reflecting the fact that disulfide anion radicals are known reducing agents.[4,36] Increases in stability of these species are indicated by smaller negative (*i.e.* more positive) potentials. While the K_ps depend on the strengths of the -S$\therefore$ S- bonds and differences in the solvation energies of the $\langle MS^{\bullet}\rangle_x$, $\langle MS^{-}\rangle_x$ and $\langle MS\therefore SM\rangle_x$ species, the $E^{o}(\langle MSSM\rangle_x/\langle MS\therefore SM\rangle_x)$s are determined by the $\langle MSSM\rangle_x$ LUMO (or σ* orbital) energies and the differences in solvation of $\langle MSSM\rangle_x$ and $\langle MS\therefore SM\rangle_x$.§ Comparison of the $E^{o}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$s for CEA, Cys, Pen and GSH shows that they indicate the same order of stability as the K_{p0} values in Table 2, namely: GSH > CEA > Cys > Pen. This is expected, since the energy of their σ* orbital is sensitive to the same inductive effects that control the strength of the σ(2)σ*(1) bond.¶ For CEA, Cys and Pen the potentials become more positive by about 0.1 V for each $-NH_3^+$ present. The fact that the apparent destabilisation of $\langle MS\therefore SM\rangle_1$ seen in the lower values of K_{p1} for CEA, Cys and Pen is not present in the E^{o} values supports the above interpretation that solvation must favour the dissociation products in equilibrium (3p1)/(−3p1). An increase in potential with protonation is also seen with GSH, but, as expected, the differences are smaller due to the larger separation of the S and $-NH_3^+$ groups. A further quantity of interest is the potential for simultaneous addition of protons to $-NH_2$ groups and electrons to the -SS- of an unprotonated disulfide. From present data the value of $E^{o}(\langle MSSM\rangle_0, 2H^+/\langle MS\therefore SM\rangle_2)$ was estimated to be −0.36 V for CEA. A similar increase of about +1 V from $E^{o}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$ in Table 3 is obtained for the other compounds.

The present $E^{o}(\langle MSSM\rangle_x/\langle MS\therefore SM\rangle_x)$ values are relevant to the stabilty of disulfide anions in proteins. In particular, $E^{o}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$ for glutathione disulfide (−1.41 V) should be a good indication of the value for the cystine residue in proteins. As pointed out above for the K_{p0}s, the difference in inductive effects of the peptide linkages and the H_2N and CO_2^- groups cause this -S$\therefore$ S- to be more stable than that of free cystine. One may also expect that -S$\therefore$ S- groups in proteins will be stabilised by neighbouring $-NH_3^+$ or other positive groups. In this regard it should be noted that $CO_2^{\bullet -}$ reduction of hen egg white lysozme, which possesses four disulfides, occurs specifically at the 6-127 -SS- linkage. It appears that here the -S$\therefore$ S- structure can be stabilised by neighbouring positive arginine groups.[10]

Disulfide anion radicals are also oxidising agents through half reactions (24p0), (24p1) and (24p2). As seen from Table 3, the reduction potentials for these half reactions for Pen are about 0.08 V higher than for CEA and Cys, and this can be attributed to the weaker bonding between the $\langle MS^{\bullet}\rangle$ and $\langle MS^{-}\rangle$ species, *i.e.* the lower K_{px} values for Pen. For all three compounds the values increase slightly for one proton addition and by about 0.04 V for diprotonation. For GSH, where inductive effects are negligible, they are independent of the degree of protonation.

Conclusions

The stability of -S$\therefore$ S- groups formed by amino thiols has been analysed in terms of their ease of dissociation into thiolate anion and thiyl radical (reaction (−3px)) and their formation by reduction of -SS- disulfide groups (reaction (2p*x*)). In the absence of protonated amino groups the reduction potential $E^{o}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$ and the strength of the -S$\therefore$ S- σ(2)σ*(1) bond increase with electron withdrawing ability of the groups attached to the S atoms. The presence of electron donating methyl groups on the alpha carbon causes a significant reduction in both parameters. Reductions in these quantities also occur when $-CO_2^-$ groups are placed on the beta carbon. It is likely that charge repulsion between the carboxylates and the negative -S$\therefore$ S-, and between themselves plays a significant role.

The protonation of amino groups beta to the S atoms causes a stepwise ~0.1 V increase in the reduction potentials, and protonation of amino groups on both beta C atoms causes a three to tenfold increase in the stability of $\langle MS\therefore SM\rangle_2$ with respect to dissociation to thiolate and thiyl radical. The smaller but similar effects in GSH, where the amino groups are too far removed from the S atoms for inductive effects to be important, appear to be caused by through space Coulombic interactions.

$E^{o}(\langle MSSM\rangle_0/\langle MS\therefore SM\rangle_0)$ for glutathione disulfide (−1.41 V) should be a good indication of the -SS- reduction potential for the cystine residue in proteins, where the inductive effects will be different from those in the free amino acid. Here too the -S$\therefore$ S- will be stabilised by nearby $-NH_3^+$ or other positive groups.

§ Since only one reactant and product appears in the reduction half reaction, in a sense the E^{o}s are a more straightforward measure of the relative stabilities of the $\langle MS\therefore SM\rangle_x$ species than the K_ps.

¶ In this regard one may note that the gas phase electron affinity of HSSH is calculated in reference 10 to be larger than that of MeSSMe.

Acknowledgements

This work was supported by NSERC of Canada and the University of Calgary. The authors are also grateful to the Notre Dame Radiation Laboratory, where the work described in references 25–29 was carried out.

References

1 M. Friedman, *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids*, *Peptides and Proteins*, Pergamon Press, New York, 1973.
2 Yu. M. Torchinskii, *Sulhydryl and Disulfide Groups of Proteins*, Translated by H. B. F. Dixon, Consultants Bureau, New York, 1974.
3 H. Sies, *Naturwissenschaften*, 1989, **76**, 56.
4 C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor and Francis, London, 1987.
5 C. von Sonntag and H.-P. Schuchmann, *Sulfur Compounds and 'Chemical Repair' in Radiation Biology.* In *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*, C. Chatgilialoglu and K.-D. Asmus, Eds., NATO ASI Series, Plenum Press, New York, 1990, pp. 409–414.
6 A. L. Lehninger, *Biochemistry*, Worth Publishers, New York, 1970.
7 A. R Bassindale and J. N. Iley, in *The Chemistry of Sulfenic Acids and Their Derivatives*, S. Patai, Ed., Chapter 4, John Wiley, New York, 1990.
8 L. Bonazzola, J. P. Michaud and J. Roncin, *J. Chem. Phys.*, 1985, **83**, 2727.
9 (*a*) M. A. Cremonini, L. Lunazzi and G. Placucci, *J. Chem. Soc., Perkin Trans. 2*, 1992, 451; (*b*) M. A. Cremonini, L. Lunazzi and G. Placucci, *J. Org. Chem.*, 1993, **58**, 3805.
10 J. Bergès, E. Kassab, D. Conte, E. Adjadj, C. Houée-Levin, *J. Phys. Chem.*, 1997, **101**, 7809.
11 T. Clark, *J. Comput. Chem.*, 1982, **3**, 112.
12 K.-D. Asmus, in *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*, C. Chatgilialoglu and K.-D. Asmus, Eds., NATO ASI Series, Plenum Press, New York, 1990, pp. 155–172.
13 J. R. Clement, D. A. Armstrong, N. V. Klassen and H. A. Gillis, *Can. J. Chem.*, 1972, **50**, 2833.
14 R. H. Bisby, R. B. Cundall, J. L. Redpath and G. E. Adams, *J. Chem. Soc., Faraday Trans. 1*, 1976, **72**, 51.
15 A. J. Elliot, F. Wilkinson and D. A. Armstrong, *Int. J. Radiat. Biol.*, 1980, **38**, 1.
16 (*a*) M. Faraggi and I. Pecht, *Isr. J. Chem.*, 1972, **10**, 1021; (*b*) M. Faraggi, J. L. Redpath and Y. Tal, *Radiat. Res.*, 1975, **64**, 452.
17 M. Z. Hoffman and E. Hayon, *J. Phys. Chem.*, 1973, **77**, 990.
18 N. N. Lichtin, J. Ogdan and G. Stein, *Biochem. Biophys. Acta*, 1972, **263**, 14; *ibid.*, 1972, **276**, 124.

19 J. R. Clement, W. S. Lin, D. A. Armstrong, G. M. Gaucher, N. V. Klassen and H. A. Gillis, *Int. J. Radiat. Biol.*, 1974, **26**, 571.
20 A. A. Iskakov and K. M. L'Vov, *Biophysics*, 1997, **42**, 1219 and papers cited therein.
21 *Oxidative Damage and Repair: Chemical, Biological and Medical Aspects*, K. J. A. Davies, Ed., Pergamon Press, New York, 1991.
22 (*a*) C. C. Winterbourn, *Free Radical Biol. Med.*, 1993**, 14**, 85; (*b*) W. H. Koppenol, *Free Radical Biol. Med.*, 1993, **14**, 91.
23 P. Wardman, *Sensitization and Protection of Oxidative Damage Caused by High Energy Radiation*, pp. 101–127, in *Atmospheric Oxidation and Antoxidants*, Vol. III, Ed. G. Scott, Elsevier, 1993.
24 P. Wardman and C. von Sonntag, *Methods Enzymol.*, 1995, **251**, 31.
25 S. P. Mezyk, *Chem. Phys. Lett.*, 1995, **235**, 89.
26 S. P. Mezyk, *J. Phys. Chem.*, 1995, **99**, 13970.
27 S. P. Mezyk, *J. Phys. Chem.*, 1996, **100**, 8295.
28 S. P. Mezyk, *J. Phys. Chem.*, 1996, **100**, 8861.
29 S. P. Mezyk, *Radiat. Res.*, 1996, **145**, 102.
30 J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, John Wiley, New York, 1961.
31 G. D. Fasman, Ed., *Handbook of Biochemistry and Molecular Biology*, *Physical and Chemical Data*, 3rd edn. CRC Press Inc., Boston, 1976, Vol. 1.
32 H. E. Laitinen and W. E. Harris, *Chemical Analysis: An Advanced Text and Reference*, 2nd edn., McGraw-Hill, New York, 1975.
33 (*a*) J. W. Purdie, H. A. Gillis and N. V. Klassen, *Can. J. Chem.*, 1973, **51**, 3132; (*b*) E. W. Wilson, Jr. and R. B. Martin, *Arch. Biochem. Biophys.*, 1971, **142**, 445.
34 D. L. Rabenstein, *J. Am. Chem. Soc.*, 1973, **95**, 2797.
35 K. K. Millis, K. H. Weaver and D. L. Rabenstein, *J. Org. Chem.*, 1993, **58**, 4144.
36 D. A. Armstrong, in *The Chemistry of Sulfur Centered Radicals*, Z. Alfassi, Ed., Chapter 2, John Wiley, New York, 1999.
37 P. S. Surdhar and D. A. Armstrong, *J. Phys. Chem.*, 1987, **91**, 6532.
38 E. Gonick, W. C. Fernelius and B. E. Douglas, *J. Am. Chem. Soc.*, 1954, **76**, 4671.
39 E. P. Serjeant and B. Dempsey, In IUPAC Chemical Data Series No. 23, *Ionisation Constants of Organic Acids in Aqueous Solution*, Pergamon Press, Oxford, 1979.
40 J. March, *Advanced Organic Chemistry*, 3rd edn., John Wiley, New York, 1985.
41 D. D. Perrin, B. Dempsey and E. P. Serjeant, *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, New York, 1981.
42 D. A. Armstrong, Qun Sun and R. H. Schuler, *J. Phys. Chem.*, 1996, **100**, 9892.
43 J. E. Packer, in *The Chemistry of the Thiol Group, Part 2*, S. Patai, Ed., John Wiley, New York, 1974, pp. 481–517.

Paper 9/01155B