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# UNPAIRED ELECTRON MIGRATION BETWEEN AROMATIC AND SULFUR PEPTIDE UNITS

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Cysteine thiyl radicals (Cys/S·) were found capable of one-electron oxidation of tyrosine. Equilibration occurred, using Cys and Gly-Tyr, with an equilibrium constant of $K_5 = 20 \pm 4$ at pH 9.15:

$$Cys/S^{\cdot} + Tyr \rightleftharpoons Cys + Tyr/O^{\cdot} \quad (5)$$

Hence the reduction potentials of these couples differ at pH 9.15 by E(Cys/S·, Cys) − E(Tyr/O·, Tyr) = 80 mV. Oxidation of Trp-Gly by Cys/S· was not detectable from pH 7 to 12. The methionyl radical cation (Met/S·N), formed via ·OH-attack on Met-Gly, reacts with Trp-Gly to generate the indolyl radical (Trp/N·). New results on intramolecular Trp/N· → Tyr/O· transitions indicate that the reaction requires direct contact between the two redox centers. Various possible pathways for migration of unpaired electrons between peptide units are compiled in a scheme.

Key words: Cysteine, thiyl radicals; electron migration; free radicals; methionyl radicals; tryptophyl radicals; tyrosyl radicals

*Abbreviations:* Cyclic dipeptides (diketopiperazines): c-$Ala_2$, c-Trp-Tyr; Radicals (examples): Cys/S· = cysteine thiyl, $Cys_2/SS^{\cdot -}$ = cystine disulfide radical anion, Met/S·Br = sulfur-bromine adduct of methionine (three-electron bonded), Trp/N· = tryptophan indolyl (neutral), Tyr/O· = tyrosine phenoxyl; TX-100 = Triton X-100.

## INTRODUCTION

Aromatic and sulfur amino acids are key functions in the migration of unpaired electrons in peptides and proteins. One-electron oxidation or reduction of these functions can be initiated in an aqueous environment in several ways, i.e. by ionizing radiation[1-11], by UV-light[2,12-16], by $CCl_4$ metabolites[17], by atmospheric pollutants such as nitrogen dioxide[18], and by peroxidizing enzymes[19,20]. An example of electron

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deficiency migration is the indolyl → phenoxyl transition (1), which has been detected in numerous peptides and proteins[4,5]:

$$\text{Trp/N}^{\cdot} + \text{Tyr} \longrightarrow \text{Trp} + \text{Tyr/O}^{\cdot} \quad (1)$$

Also of considerable interest is the observation that electron addition to peptide carbonyls can result in deamination by electron transfer to protonated amino groups[6]. In cyclic and N-acetylated peptides the electron adduct becomes stabilized and capable of electron transfer to thiols and disulfides[7,8], e.g.

$$\text{c-Ala}_2^{\cdot -} + \text{Cys}_2 \longrightarrow \text{c-Ala}_2 + \text{Cys}_2/\text{SS}^{\cdot -} \quad (2)$$

The $\text{Cys}_2/\text{SS}^{\cdot -}$ radical anion still behaves as a reductant, capable of electron transfer to $O_2$[11]. Dissociation of the anion, however, leads to the electron deficient thiyl radical[9-11,16],

$$\text{Cys}_2/\text{SS}^{\cdot -} (+\text{H}^+) \rightleftharpoons \text{Cys} + \text{Cys/S}^{\cdot} \quad (3)$$

The equilibrium (3) may present an important crossover between reductive and oxidative transitions.

The present investigation was aimed at demonstrating interactions between thiyl radicals and aromatic amino acids. New data are also presented on transfer of electron deficiency between Met, Trp and Tyr.

## MATERIALS AND METHODS

Peptides, unless stated, were of L-isomeric conformation: $(\text{Cys-Tyr})_2$, Gly-Trp-Tyr-Gly and c-D-Trp-L-Tyr from Bachem (Bubendorf, Switzerland), all other peptides from Serva (Heidelberg, FRG). Radical transitions were initiated in aqueous solution at about 20°C by primary water radiolysis products,

$$\text{H}_2\text{O} \xrightarrow{\text{radiation}} {}^{\cdot}\text{OH}, e_{aq}^{-}, \text{H}^{\cdot} \text{ etc} \quad (4)$$

or by secondary radicals, as previously described[4,5], using the Paterson Laboratories pulse radiolysis facility[21]. The dose per pulse ($< 2$ μs) was chosen so as to produce less than one radical per ten peptide molecules (to reduce undesired radical-radical reactions). Solutions were freshly prepared for each experiment with redistilled water and flushed gently either with $N_2O$, to convert $e_{aq}^{-}$ into ${}^{\cdot}\text{OH}$[1], or when $e_{aq}^{-}$ was wanted as one-electron reductant, with Ar.

## RESULTS

Pulse radiolysis of deaerated solutions of the cystinyl peptide $(\text{Cys-Gly})_2$, in the presence of Gly-Tyr and t-butanol (as scavenger for ${}^{\cdot}\text{OH}$), produced a broad absorption around 410 nm which decayed within few μs, consistent with formation of $\text{R}_2/\text{SS}^{\cdot -}$ species by $e_{aq}^{-}$[9,10], and decay according to reaction (3). The thiyl $\text{R/S}^{\cdot}$ is transparent at 410 nm[10], and the reverse reaction (3) is negligible at the low doses applied. As shown in Figure 1 (insert) a secondary absorption grew in after the disappearance of $\text{R}_2/\text{SS}^{\cdot -}$, the build-up rate being proportional to the Gly-Tyr concentration. The secondary absorption spectrum (Figure 1) resembled the 385/405 nm

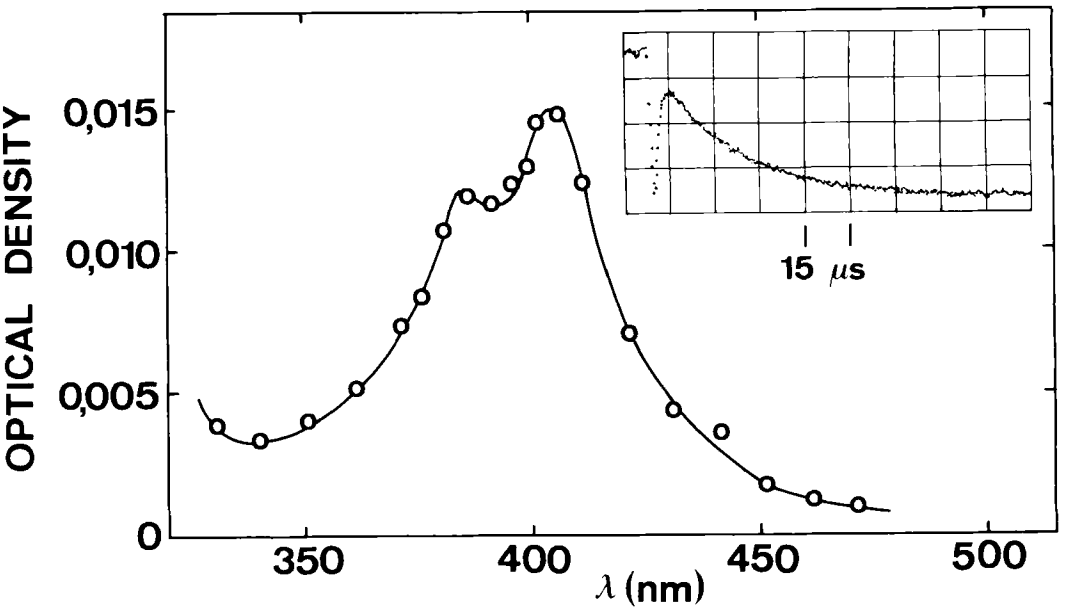



FIGURE 1 Generation of phenoxyl by the reaction of a thiyl with Gly-Tyr. The reactions (3)–(5) were initiated with $e_{aq}^-$ by 1.6 Gy pulse radiolysis of a deaerated aqueous solution containing 3.2 mM $(Cys\text{-}Gly)_2$, 3.2 mM Gly-Tyr and 0.8 M t-butanol at pH 9.0 (spectrum at 120 μs). Inserted is a time profile of the optical transmission (10 cm cell) at 405 nm.

phenoxyl absorption; it was not produced in the absence of $(Cys\text{-}Gly)_2$. This result evidently demonstrates phenol oxidation by the thiyl radical,

$$Cys/S^{\cdot} + Tyr \rightleftharpoons Cys + Tyr/O^{\cdot} \qquad (5)$$

The yield of $Tyr/O^{\cdot}$, obtained with $\epsilon_{405} = 3200\ M^{-1}\ cm^{-1}$[22], is 0.29 μM/Gy, indicating complete transfer (the initial yield of $e_{aq}^-$ is 0.29 μM/Gy). Rate constants $k_5$ ($Cys/S^{\cdot}$-Gly + Gly-Tyr) increase with pH, as shown in Table I. When the thiyl was generated in cysteine solutions by reaction with $Br_2^{\cdot -}$ or $NO_2^{\cdot}$ [18], incomplete tyrosine oxidation was observed. From equilibration of $Cys_2/SS^{\cdot -}$ absorption at 480 nm (where $Tyr/O^{\cdot}$ is transparent) in presence of various Gly-Tyr concentrations (Figure 2) an equilibrium constant $K_5$ was obtained for reaction (5): $K_5/K_3 = 3.3 \times 10^{-3}$ M at pH 9.15, or $K_5 = 20 \pm 4$ when adopting the value of $K_3$ at this pH of $(6 \pm 1) \times 10^3\ M^{-1}$[11]. This result enables an estimate to be given for the one-electron reduction potentials promoting reaction (5) at pH 9.15: E(Cys/S·, Cys) − E(Tyr/O·, Tyr) = $0.059 \log K_5 = 80$ mV.

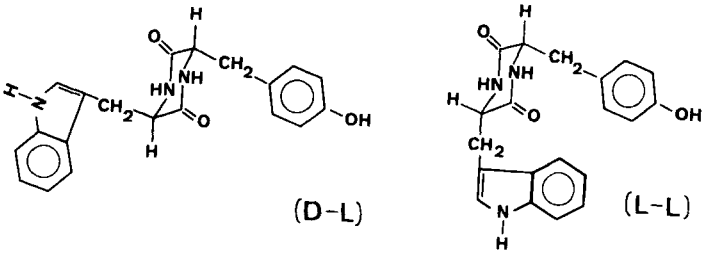
Oxidation of tryptophan (4 mM Trp-Gly) by $Cys/S^{\cdot}$ was not detectable in the range pH 7 to 12. The transient 400 nm absorption, formed by reaction of ·OH with Met-Gly[4] and assigned[23] to a three-electron bonded S.∴N radical cation (here denoted $Met/S^{\cdot}N$), was capable on the other hand of one-electron oxidation of tryptophan. This is demonstrated in Figure 3 by the secondary build-up, in presence of Trp-Gly, of the characteristic 510 nm indolyl absorption,

$$Met/S^{\cdot}N + Trp \longrightarrow Met + Trp/N^{\cdot} \qquad (6)$$

TABLE I
Rate Constants for Radical Transitions in Peptide Model Systems[a]

| Transition | Model System | pH | Rate Constant |
|---|---|---|---|
| Cys/S$^{\cdot}$ → Tyr/O$^{\cdot}$ | $(Cys\text{-}Gly)_2$ + Gly-Tyr[b] | 5.9 | $1.3 \times 10^6\ M^{-1}\ s^{-1}$ |
| | | 7.8 | $6.3 \times 10^6\ M^{-1}\ s^{-1}$ |
| | | 9.0 | $1.1 \times 10^7\ M^{-1}\ s^{-1}$ |
| | | 10.1 | $5.8 \times 10^7\ M^{-1}\ s^{-1}$ |
| | Cys + Gly-Tyr[c] | 9.2 | $2.5 \times 10^7\ M^{-1}\ s^{-1}$ |
| Met/S$^{\cdot}$N → Trp/N$^{\cdot}$ | Met-Gly + Trp-Gly[d] | 6.8 | $4.0 \times 10^7\ M^{-1}\ s^{-1}$ |
| Trp/N$^{\cdot}$ → Tyr/O$^{\cdot}$ | c-Trp-Tyr,[e] (L-L)[f] | 7.7 | $5.8 \times 10^4\ s^{-1}$ |
| | c-Trp-Tyr,[e] (D-L)[f] | 7.3 | $2.0 \times 10^3\ s^{-1}$ |
| | Gly-Trp-Tyr-Gly[e] | 7.3 | $2.0 \times 10^4\ s^{-1}$ |

[a] Present results (references to previous results are given in Scheme I); intramolecular transitions in $s^{-1}$ (independent of peptide concentration), intermolecular transitions in $M^{-1}\ s^{-1}$. [b] Initiated by $e_{aq}^-$, see text. [c] Initiated by $NO_2^{\cdot}$ or $Br_2^{\cdot -}$. [d] Initiated by $^{\cdot}OH$. [e] Initiated by $N_3^{\cdot}$. [f] In 1% (w/w) TX-100; structures:



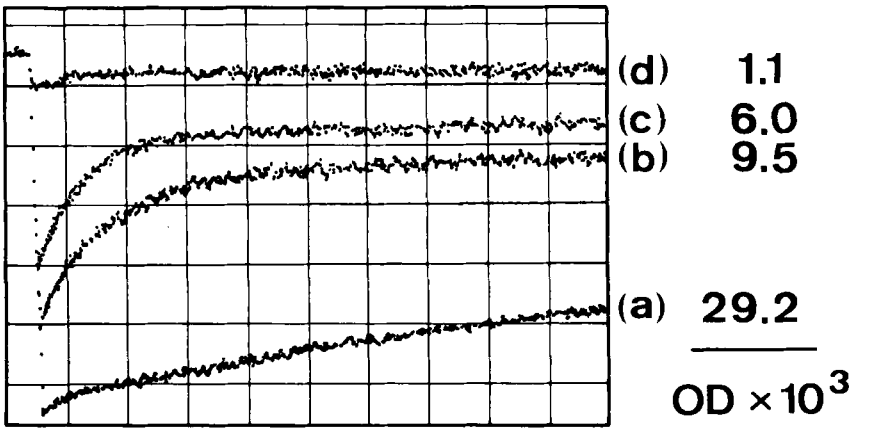




FIGURE 2 Time profiles (at 480 nm and 20 μs/division), showing the equilibration of reaction (5) at pH 9.15. The results were obtained with $Br_2^{\cdot -}$ as oxidant, by 1.65 Gy pulse radiolysis of $N_2O$-saturated solutions of 50 mM KBr in presence of: (a) 2 mM Cys, (b) 2 mM Cys + 3 mM Gly-Tyr, (c) 2 mM Cys + 6 mM Gly-Tyr, (d) 3 mM Gly-Tyr. OD = optical density at equilibrium (predominantly due to $Cys_2/SS^{\cdot -}$ absorption).

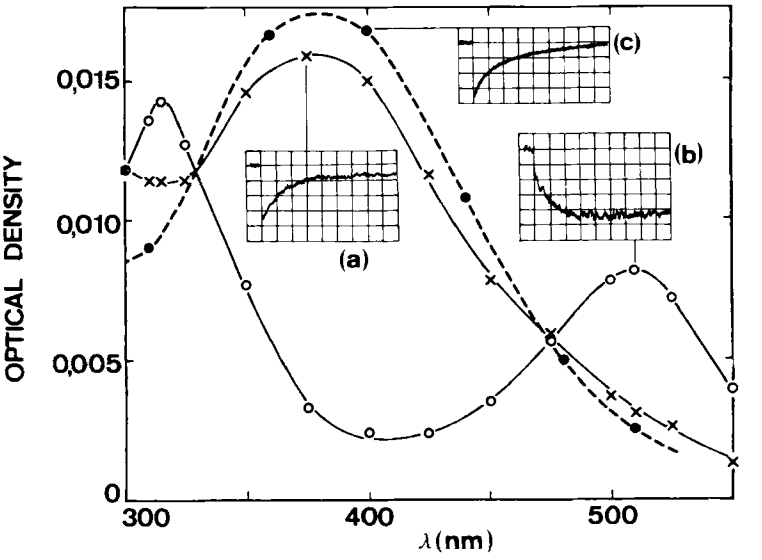



FIGURE 3 Transient Met/S·N absorption (×), and its transformation into Trp/N· absorption (o). The reaction was initiated with ·OH by 3.2 Gy pulse radiolysis of a $N_2O$-saturated aqueous solution containing 14 mM Met-Gly and 2 mM Trp-Gly in 7 mM phosphate buffer, pH 6.8. Spectra at 1 μs (×) and 50 μs (o) after the pulse (2.5 cm cell), time profiles (10 μs/division) at 375 nm (a) and 510 nm (b). The dotted spectrum at 10 μs (•) and the 400 nm trace (c, 500 μs/division) were obtained in the absence of Trp-Gly.

The yield of Trp/N·, obtained with $\epsilon_{510} = 2000\ M^{-1}\ cm^{-1}$[2], is 0.50 μM/Gy and approaches the yield of ·OH of 0.58 μM/Gy. The rate constant of the transfer (Table I) is not greatly different from that of the corresponding Met/S·Br → Trp/N· transition ($1.1 \times 10^8\ M^{-1}\ s^{-1}$) previously described[5].

Table I also includes new results on Trp/N· → Try/O· transitions within peptides with adjacent Trp and Tyr units. As can be seen, the transfer in c-Trp-Tyr is slowed down by a factor of 30, when going from the L-L to the D-L enantiomer, and in linear peptides by a factor of 3, when going from Trp-Tyr ($k = 6 \times 10^4\ s^{-1}$[4]) to Gly-Trp-Tyr-Gly.

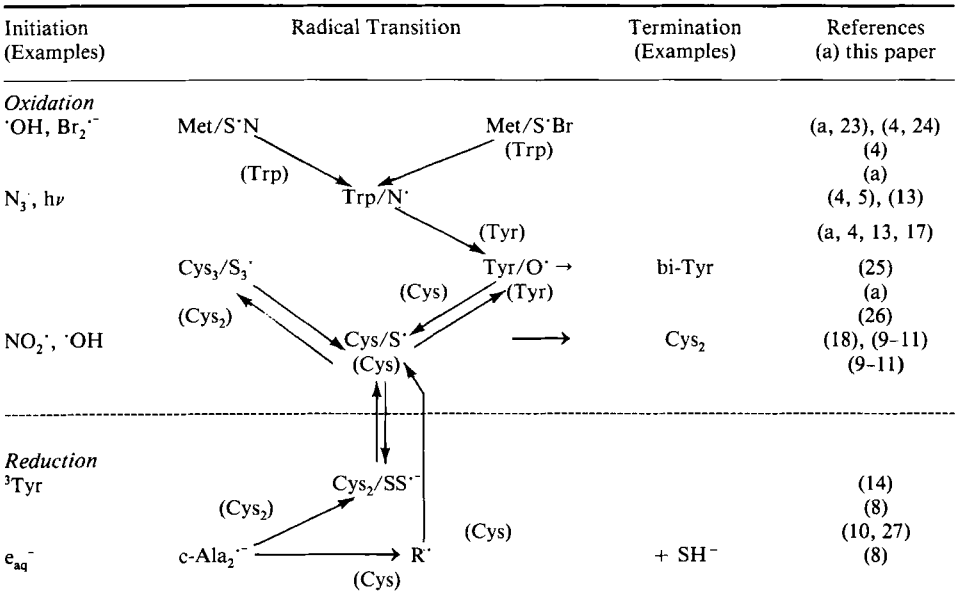
Various pathways of unpaired electron migration, as now established in peptide model systems, are compiled in Scheme I.

## DISCUSSION

The Met/S·N → Trp/N· transfer (Scheme I, Figure 3) is favoured in Met-Gly, due to stabilization of the methionyl by the amino group[23] and the Gly unit[4]. In free methionine where these factors are absent the oxidizing Met/S·N decays by fast decarboxylation, thereby generating a reducing α-amino radical[28]. Surprisingly, the Met-α-amino radical was also found capable of oxidizing Cys to Cys/S·[28]; this latter process may, however, primarily involve Cys reduction, followed by the R·-induced oxidation of Cys depicted in Scheme I[10,27].

Intramolecular Trp/N· → Tyr/O· transitions were previously found to proceed faster the more flexible the peptide[4]. The very slow transfer in c-D-Trp-L-Tyr

SCHEME I
Selected Radical Transitions Between Peptide Units in Neutral Aqueous Solution




(Table I) furthermore illustrates that the reaction requires a direct contact between the two reaction centers, this being sterically restricted particularly in the D-L enantiomer, as can be shown with molecular models. There has been little evidence[4] for equilibration of reaction (1) in neutral solution. However, the reverse transition, Tyr/O· → Trp/N·, has been detected in strongly alkaline or acid solutions where the reduction potential of the phenoxyl becomes more positive than that of the indolyl[29].

The Cys/S· ↔ Tyr/O· equilibrium (5) enables us to estimate the thiyl reduction potential: with the data derived from Figure 2 and E(Tyr/O·, Tyr) = 0.60 ± 0.05 V at pH 13 (cf. 29) we obtain E(Cys/S·, Cys) = 0.73 ± 0.05 V at pH 13 and E(Cys/S·, Cys) ≃ E(Tyr/O·, Tyr) below pH 8 (the pK of Cys). This means that equilibration below pH 8 occurs with $K_5 \simeq 1$ (or even lower). The reverse of reaction (5) appears in fact to predominate in neutral solution, as shown by the finding that Tyr/O· termination by bi-Tyr coupling was strongly inhibited by Cys at pH 7[25]. The coupling of Cys/S· to $Cys_2$, as depicted in Scheme I, might therefore be a most important reaction terminating various pathways of unpaired electron migration between peptide units in neutral solutions.

Charge migrations as depicted in Scheme I are likely to proceed also in proteins. In fact this has been demonstrated in the case of Trp/N· → Tyr/O· and Met/S·Br → Tyr/O· transitions[5,30]. Of particular interest is the disulfide·−/thiyl equilibrium (3) which involves disulfide rupture and mediates between reductive and oxidative electron transfer. The characteristic 410 nm absorption of $Cys_2/SS^{\cdot -}$ has certainly been generated in numerous proteins[31,32]. Its decay, followed by possible Cys/S· → Tyr/O· transfer has not previously been recognized, however, probably due to the unfavourable overlap of the absorptions of $Cys_2/SS^{\cdot -}$ and Tyr/O·. There is evidence also for an interaction of Tyr/O· with cystine[30], possibly leading to disulfide radical

cations. Thiyls formed in proteins by reductive or oxidative pathways may eventually terminate by intra- or intermolecular S-S-coupling[33].

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