Reversibility of Charge Transfer between Tryptophan and Tyrosine

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Whereas tryptophan radicals oxidise tyrosine over a wide range of pH values, the one-electron reduction potential of the electron-deficient tryptophan radical at pH 7 is only 0.093 V more positive than that of the corresponding tyrosine radical, so that the reaction proceeds in reverse in strongly acid and alkaline solution.

Several earlier papers have demonstrated that, if formed in a peptide or a protein, the one-electron deficient radical of tryptophan, Trp·, can often transfer its electron deficiency to tyrosine, producing a radical of the phenoxyl type, TyrO· [reaction (1)]. The one-electron reduction potentials of the tryptophan and tyrosine radicals were estimated to be about 1 and 0.5 V respectively at pH 7.[1] It follows from the pK_a of the tryptophan radical, 4.3,[2] and the pK_a of the phenoxylic group of

$$\text{Trp}\cdot + \text{TyrOH} \longrightarrow \text{TrpH} + \text{TyrO}\cdot \qquad (1)$$

tyrosine, 10.1, that the one-electron reduction potentials of the tryptophan and tyrosine radicals must become progressively closer together for two or three pH units below 4.3 and above 10.1 than they are in the neutral region, although they could not meet at accessible pH values if the estimated reduction potentials were to be correct. We have now found that transfer of electron deficiency proceeds in the opposite direction to reaction (1) in acid and/or alkaline solution. This shows that the reduction potentials of the tryptophan and tyrosine radicals must be closer together than previously thought.

To examine the direction of the transfer at acid pH, we have prepared oxygen-saturated solutions containing 10^{-3} mol dm^{-3} L-tyrosine, 10^{-4} mol dm^{-3} L-tryptophan, and 0.5 mol dm^{-3} sulphuric acid, and delivered single 50 ns pulses (5 Gy) of ~10 MeV electrons to them. Under these conditions we expect almost all OH radicals to attack tyrosine, yielding radicals of the phenoxyl type after acid–base catalysed water elimination.[3] Hydrogen atoms would be taken up to yield unreactive HO_2. Observations were made of the tyrosine radicals at their absorption maximum of 405 nm and of tryptophan radical cations at their absorption maximum in acid solutions of 570 nm. We have found that the tyrosine radicals disappeared and the tryptophan radicals were formed with the same half-life, 10 μs, from which the rate constant for the transfer becomes 7×10^8 dm^3 mol^{-1} s^{-1}. There was also good evidence for reaction (2) at pH 1.1, but beyond pH 2 tryptophan radicals gave rise to tyrosine radicals as previously observed.[4] Distinct evidence of reaction (2) was also obtained from pulse radiolysis of air-saturated solutions of pepsin (2 mg/ml) at pH 1, but no evidence of transfer in either direction was obtained at pH 2. Earlier studies with pepsin had shown that at pH 5.8 tryptophan radicals gave rise to tyrosine radicals.[1]

$$\text{TyrO}\cdot + \text{TrpH} + \text{H}^+ \longrightarrow \text{TyrOH} + \text{TrpH}^{+\cdot} \qquad (2)$$

$$\text{TyrO}\cdot + \text{TrpH} \longrightarrow \text{TyrO}^- + \text{Trp}\cdot + \text{H}^+ \qquad (3)$$

To examine transfer in alkaline solution, we have delivered pulses to N_2O-saturated solutions containing 10^{-2} mol dm^{-3} L-tyrosine, 3×10^{-4} mol dm^{-3} L-tryptophan, and 3 mol dm^{-3} sodium hydroxide. Tyrosine radicals examined at 405 nm were found to disappear at the same rate as tryptophan radicals, examined at 510 nm, were formed, half-life 14 μs [reaction (3)]. The rate constant for the transfer was 1.65×10^8 dm^3 mol^{-1} s^{-1}. Slight evidence of transfer was seen at pH 12.5, but in N_2O-saturated solutions containing 5×10^{-2} mol dm^{-3} L-tryptophan and 5×10^{-3} mol dm^{-3} L-tyrosine (1 mol dm^{-3} azide) there was distinct disappearance of tryptophan radicals

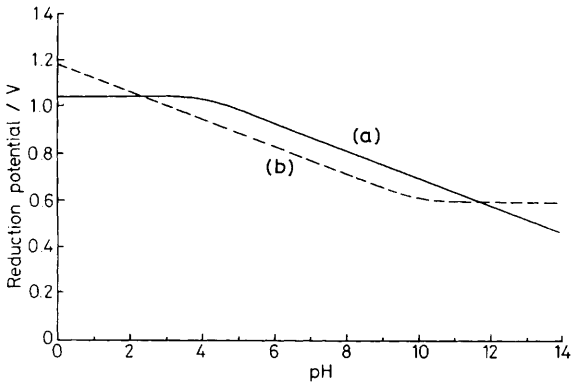

Figure 1. One electron reduction potentials of electron-deficient (a) tryptophan and (b) tyrosine radicals as a function of pH.

and formation of tyrosine radicals at pH 10 and 10.5 and possibly even at pH 11.

Neither in acid nor in alkaline solutions was it possible using the free amino acids to observe a convincing equilibrium between tyrosine and its radical and tryptophan and its radical, since except at the extremes of pH, rates of transfer were slow compared with the rates of competing reactions. However from visual inspection of traces obtained at pH 0, 1.1, 2.05, 3.6, 10.5, 12.5, 13.5, and 14.5, and knowing the pK_a values of the tryptophan radical and of tyrosine, the one-electron reduction potential of the tryptophan radical in the pH range ~5—9 could be estimated to be between 0.07 and 0.13 V more positive than the one-electron reduction potential of the tyrosine radical.

As found for transfer in the opposite direction, rates of transfer were faster when the tryptophan and tyrosine were joined by a peptide bond. This made it possible to determine an accurate equilibrium constant for the peptides. To do this, N_2O-saturated solutions were prepared containing 10^{-4} mol dm^{-3} L-tryptophyl-L-tyrosine and 1 mol dm^{-3} azide at five pH values in the range 9–13. At pH 9 there was essentially complete transfer of the electron deficiency from tryptophan to tyrosine as described previously.[4] At pH 13 there was essentially complete transfer of the electron deficiency from tyrosine to tryptophan, with a first order rate constant of about 2×10^5 s^{-1}. Equilibration was seen at intermediate pH values. The equilibrium constant was obtained most accurately from the experiment at pH 12.1, from which the constant K expressed by equation (4) was found to be 1.7 ±

$$K = [\text{Trp}^{\bullet}\text{–TyrO}^{-}]/[\text{TrpH–TyrO}^{\bullet}] \quad (4)$$

10% at this pH, *i.e.*, the one-electron reduction potential of the tryptophan radical is 0·014 ± 0·002 V less positive than that of the tyrosine radical. Assuming pK_a values for the tryptophan radical and for tyrosine of 4.3 and 10.1 respectively, the one-electron reduction potential of the tryptophan radical is then 0.093 V more positive than that of the tyrosine radical in the pH range ~ 5—9, consistent with the value derived from visual inspection as described above. From a correlation of the one-electron reduction potentials of substituted phenoxyl radicals with Hammett constants, the potential for the tyrosine radical has been estimated[5] as 0.60 ± 0.05 V at pH 13. Using the above figures, the value for the tryptophan radical then becomes 0.87 ± 0.1 V at pH 7, in full agreement, but more accurate than the previous value of 0.98 ± 0.2 V derived from completely independent considerations.[1]

The variation of the reduction potentials with pH is shown in Figure 1. For tryptophan, the assumption has been made that the radical does not have a carboxy group with a pK_a different from that in the parent. If this were to be the case, the curve in the acid region would differ from that shown. The curve in the acid region would also differ if the pK_a of the tryptophan radical were to differ from 4.3, as found for pepsin[6] and perhaps lysozyme.[7]

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