Long-Range Electron Transfer between Tyrosine and Tryptophan in Peptides†

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Abstract: The indolyl radical, formed in the one-electron oxidation of the tryptophan side chain, oxidizes the tyrosine side chain to the phenoxy radical. Since both amino acid radicals absorb in the visible region, this one-electron transfer can be monitored as an absorbance change. We report the rate constants for this intramolecular process in a variety of small peptides that contain both tyrosine and tryptophan, and we find that the distance dependence of the electron transfer across the peptide spacer -(proline)$_n$- may correlate with the redox potential difference between the electron donor/acceptor pair. As the distance between aromatic amino acid residues increases in the peptide tyrosinyl(prolinyl)$_n$-tryptophan, the decrease in rate constant is small, a result that may be of some significance in understanding physiologically important electron transfer in proteins.

The early experimental evidence for long-range electron transfer (LRET)[1] in proteins and polypeptides[2,3] has been verified by the many observations of LRET between donor/acceptor redox centers with known (crystallographic) separation distances.[4] As part of a program to unravel the mechanistic basis of LRET in proteins, we have begun a systematic investigation of the intramolecular tyrosine to tryptophan radical (Trp$^\bullet$) electron transfer first reported by Prütz and co-workers[3b] as part of the reaction series in eq 1-3,

$$e_{aq}^{\bullet-} + N_2O \xrightarrow{H_2O} OH^\bullet + N_2$$
$$OH^\bullet + N_3^- \rightarrow OH^- + N_3^\bullet \quad (1)$$

$$N_3^\bullet + \text{TrpH-X-TyrOH} \rightarrow {}^\bullet\text{Trp-X-TyrOH} + N_3^- + H^+$$

or (2)

$$N_3^\bullet + \text{TyrOH-X-TrpH} \rightarrow \text{TyrOH-X-Trp}^\bullet + N_3^- + H^+$$

$$^\bullet\text{Trp-X-TyrOH} \rightleftharpoons \text{TrpH-X-TyrO}^\bullet$$

or (3)

$$\text{TyrOH-X-Trp}^\bullet \rightleftharpoons \text{TyrO}^\bullet\text{-X-TrpH}$$

where the pulse radiolytically generated primary radicals, the hydrated electron ($e_{aq}^{\bullet-}$) and hydroxyl radical (OH$^\bullet$), are converted by the two reactions of (1) into the azide radical ($N_3^\bullet$) within the first microsecond after the pulse. The azide radical oxidizes the indolyl side chain of tryptophan (TrpH) to the neutral tryptophan radical approximately 10 times more rapidly than it oxidizes the side chain of tyrosine (TyrOH), and this preferential oxidation in (2) sets up the one-electron transfer of (3).[5] We report here the rate constants for the intramolecular electron transfer (3) in a variety of peptides and compare our -(Pro)$_n$- results with literature data obtained by others using different redox donor/acceptor pairs attached to oligoproline.

Results and Discussion

Because the tryptophan side chain has greater reactivity toward the azide radical than does the tyrosine side chain, one-electron indole oxidation is the predominant reaction with peptides that contain both tryptophan and tyrosine.[3b] This initial and rapid Trp$^\bullet$ formation, seen as an absorbance increase at the wavelength maximum of 510 nm and occurring with a rate constant of approximately 5×10^9 M^{-1} s^{-1} for all the peptides tested, is followed by a slower Trp$^\bullet$ reduction and concomitant tyrosine oxidation, seen as an absorbance decrease at 510 nm together with an absorbance increase at 410 nm due to formation of TyrO$^\bullet$. (Figure 1: On careful perusal of the 410-nm traces in Figure 1b one can detect the smaller initial rapid and competitive formation of tyrO$^\bullet$ from the azide radical.) Three pieces of experimental evidence show that this is a single reaction. There is an isosbestic point observed during the reaction (Figure 2), the first-order electron-transfer rate constant is the same whether measured at 410 or 510 nm, and on the basis of the reported extinction coefficients of TyrO$^\bullet$ and Trp$^\bullet$[6] we can measure reaction stoichiometries of 1:1. For example, in the reaction shown in Figure 2 there is 1.0 ± 0.1 mol of tyrosine oxidized per mole of Trp$^\bullet$ lost. We have collected in Table I the measured electron-transfer rate constants for this single-electron-transfer reaction in a number of peptides.

Before interpreting the results of Table I in terms of intramolecular processes, we looked, in a few cases, for intermolecular electron transfer and radical decay contributions to the observed reactions. Variation of a first-order rate constant on peptide concentration is a test for an intermolecular contribution to the electron transfer. With those peptides that contain no proline, we found no dependence of k_{app} on concentration. Hence, these reactions are solely intramolecular, or they also contain a slow intermolecular reaction with a signate concentration dependence that falls within the measurement uncertainty. With the peptides containing proline, where the measured first-order rate constants are lower (Table I), we did observe small linear concentration dependencies when we sought them. In these cases we calculated first-order intramolecular rate constants and second-order intermolecular rate constants (Table I) from the nonzero intercepts and the slopes, respectively, of the linear k_{app} versus concentration plots. Since those intermolecular rates we measured were so slow

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(1) Abbreviations used are as follows: $e_{aq}^{\bullet-}$, the hydrated electron; OH$^\bullet$, the hydroxyl radical; LRET, long-range electron transfer; Trp$^\bullet$ and TrpH$^{\bullet+}$, the indolyl neutral and cationic radicals of tryptophan; TyrO$^\bullet$ and TyrOH$^{\bullet+}$, the neutral and cationic phenoxy radicals of tyrosine.

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(5) The reader should be careful to note that there is a net transfer of a single proton in this reaction. This is due to the fact that both TrpH and TyrOH are protonated at pH 7, while the pK_a of TrpH$^{\bullet+}$ and TyrOH$^{\bullet+}$ are 4.3 and <0, respectively. Bent, D. V.; Hayon, E. *J. Am. Chem. Soc.* **1975**, *97*, 2612-2619. Dixon, W. T.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 1221-1230.

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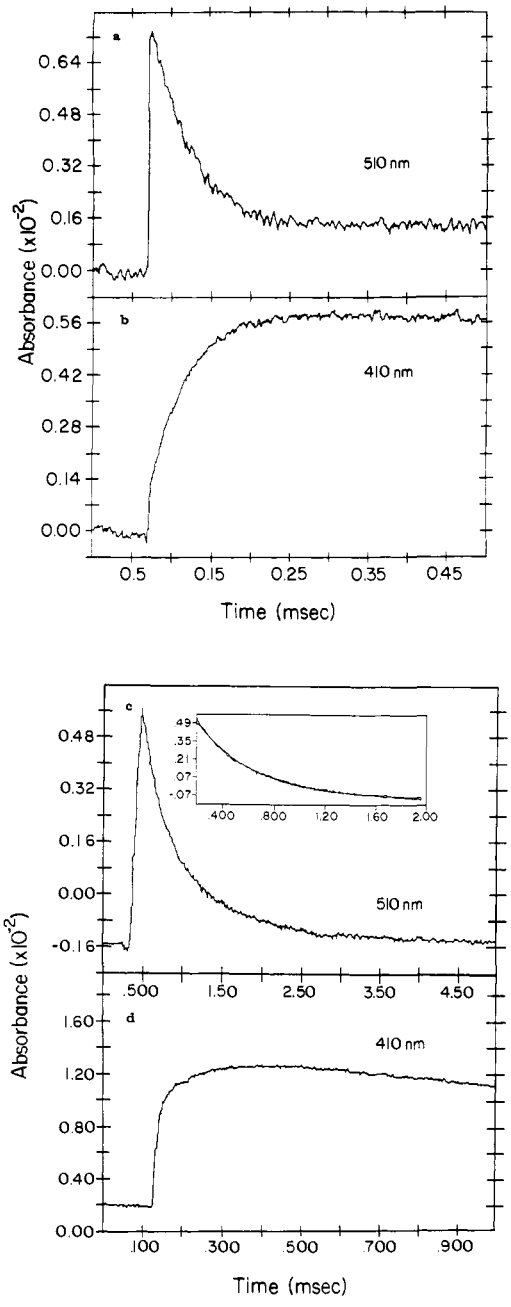

Figure 1. One-electron transfer between tyrosine and tryptophan in peptides. (a) TyrOH-Glu-TrpH: decay of Trp•. The trace can be separated into two phases: an initial absorbance increase at 510 nm due to the preferential N_3• oxidation of TrpH to Trp• and a subsequent absorbance decay due to the one-electron reduction of Trp• to TrpH. (b) TyrOH-Glu-TrpH: formation of TyrO•. After a small increase at 410 nm because of less favored N_3• oxidation of TyrOH to TyrO•, there is a subsequent first-order absorbance increase due to TyrOH oxidation to TyrO• and occurring simultaneously with the Trp• reduction to TrpH shown in panel a. (c) TrpH-Pro-Pro-Pro-TyrOH: decay of Trp•. The reaction measured at 510 nm is similar to that of panel a, with the exception of the additional slow second-order Trp• decay. In the inset we present the time course of the electron-transfer reaction starting right after the pulse. Laid over the experimental curve is the first-order decay curve calculated from the constants obtained in a nonlinear least-squares best fit. (d) TrpH-Pro-Pro-Pro-TyrOH: formation of TyrO•. The same kinetics as observed in panel b except for the now apparent slow absorbance decrease at 410 nm due to second-order radical decay. The concentrations of TyrOH-Glu-TrpH, Trp-Pro-Pro-Pro-TyrOH, and TrpH-Pro-Pro-TyrOH were 1 mM, 1 mM, and 250 μM, respectively. Otherwise, the reaction conditions were identical with N_3^- and N_3• concentrations at 0.1 M and ca. 1 μM in N_2O-saturated aqueous solutions with 5 mM phosphate, pH 7.0, 25 °C.

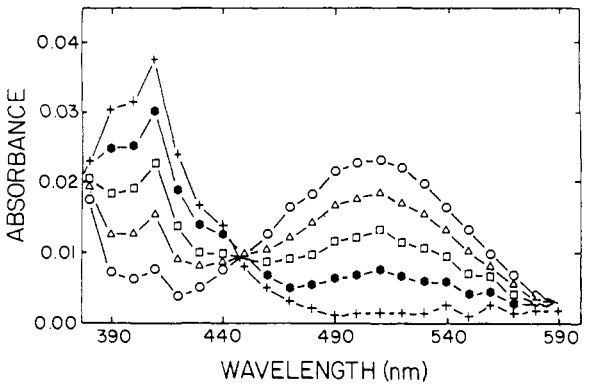

Figure 2. Spectral change during the one-electron transfer in the peptide TrpH-Pro-Pro-TyrOH. The conditions were close to those of Figure 1. The individual points were obtained from time profiles such as those of Figure 1 to construct the absorbance spectra at the following times: 2 μs, ○; 50 μs, △; 100 μs, □; 200 μs, ●; 500 μs, +.

relative to the intramolecular rates, we found it unnecessary to determine concentration dependencies for all the peptides of Table I; instead we maintained each peptide's concentration sufficiently low so that the measured rate constant was that of the intramolecular process within the measurement uncertainties. For comparison, Table I also includes the estimated second-order rate constant for electron transfer between the free tyrosine and tryptophan amino acids. This rate constant, obtained with a large uncertainty, is comparable to the observed peptide intermolecular rate constants.

Slow second-order radical decays, the rate constants of which are in the last column of Table I, occur after the intramolecular electron transfer. Because radical concentrations were kept at ≤ 1 μM, electron-transfer equilibrium was reached before appreciable radical loss. For example, $t_{1/2}$ in Tyr-$(Pro)_3$-Trp, with the slowest first-order electron transfer of all the peptides, is approximately 0.35 ms; the first half-life for the radical decay of this same peptide is ≥ 3.7 ms. Thus, the reported first-order electron-transfer rate constants are not compromised seriously by radical decay overlap. The more usual result, a clean separation between electron transfer and radical decay, is seen in Figure 1a,b for the case of Tyr-Glu-Trp. In Figure 1c,d are examples of slight overlap between radical decay and electron transfer during the slower reactions in Trp-Pro-Pro-Pro-Tyr and Trp-Pro-Pro-Tyr respectively; at longer times there is an obvious, albeit slow, loss of absorbance at both 510 and 410 nm. The separation is, however, still sufficient so as to permit a reliable estimate of the electron-transfer equilibrium position.

We can now interpret the data of Table I. First, the intramolecular electron transfer is faster when tryptophan is N-terminal and tyrosine C-terminal than when the order is reversed. A possible explanation for this difference is a change in redox potential when the amino acid is located at the N- versus C-terminal end. The redox potentials for the indolyl and phenol side chains of the free amino acids are uncertain, with values reported between ca. 0.70 and 1.1 V (vs NHE) at pH 7 for both.[7] Although not prepared here to dip an oar into these troubled waters, we have attempted to estimate the redox potential differences between the two amino acids when they are tied to the same peptide, (H_2N)Tyr-X-Trp(COOH) or (H_2N)Trp-X-Tyr(COOH). As evidenced by the differential pulse polarography results of Figure 3, the electrochemical properties of the peptides do depend on the tyrosine and tryptophan order. With tryptophan N-terminal and tyrosine C-terminal, we find two distinct polarographic peaks, and estimated TyrO•/TyrOH Trp•/TrpH differences of ca. 0.2 V[8]

(7) Butler, J.; Land, E. J.; Prütz, W. A.; Swallow, A. J. *Biochim. Biophys. Acta* **1982**, *705*, 150–162. Butler, J.; Land, E. J.; Prütz, W. A.; Swallow, A. J. *J. Chem. Soc., Chem. Commun.* **1986**, 348–349. Jovanovich, S. V.; Harriman, A.; Simic, M. G. *J. Phys. Chem.* **1986**, *90*, 1935–1939. Harriman, A. *J. Phys. Chem.* **1987**, *91*, 6102–6104. Merényi, G.; Lind, J.; Shen, X. *J. Phys. Chem.* **1988**, *92*, 134–137. Faraggi, M.; Weinraub, D.; Broitman, F.; DeFelippis, M. R.; Klapper, M. H. *Radiat. Phys. Chem.* **1988**, *32*, 293–297.

Table I. Electron Transfer between Tryptophan and Tyrosine

compound		k_{intra},[a] s^{-1}	k_{inter}, s^{-1} M^{-1}	$\delta\epsilon_m{}^7$,[e] mV	$\delta\epsilon_m{}^7$,[f] mV	k_{decay},[g] s^{-1} M^{-1}
Trp + Tyr			$\sim 5 \times 10^5$[b]			
Trp-Tyr		7.4×10^4 (7.3×10^4)[c]	nd[d]	>80	190	7.0×10^8
	Tyr-Trp	6.7×10^4 (5.4×10^4)[c]		70	≤70	7.2×18^8
	Tyr-Lys-Trp	2.6×10^4		60	≤70	4.6×10^8
Trp-Gly-Tyr		6.1×10^4 (5.1×10^4)[c]		>80	190	5.4×10^8
	Tyr-Gly-Trp	2.4×10^4		75	≤70	5.7×10^8
Trp-$(Gly)_2$-Tyr		2.9×10^4 (2.4×10^4)[c]		>80	215	4.8×10^8
Trp-Glu-Tyr		5.9×10^4		>80	155	4.2×10^8
	Tyr-Glu-Trp	2.1×10^4	nd	65	≤70	3.9×10^8
	Tyr-$(Glu)_2$-Trp	2.7×10^4	nd	65	≤70	1.8×10^8
	Tyr-$(Glu)_3$-Trp	1.5×10^4	nd	70	≤70	1.2×10^8
	Tyr-Pro-Trp	0.72×10^4	10×10^5	55	≤70	4.3×10^8
Trp-$(Pro)_2$-Tyr		0.67×10^4		>80	205	4.0×10^8
	Tyr-$(Pro)_2$-Trp	0.32×10^4	8×10^5	55	≤70	3.5×10^8
Trp-$(Pro)_3$-Try		0.23×10^4		>80	215	2.9×10^8
	Tyr-$(Pro)_3$-Trp	0.20×10^4	5×10^5	55	≤70	2.7×10^8

[a] The estimated errors of all of our reported first-order rate constants fall in the range of 10–20%. Because of the size of the estimated equilibrium constants and the magnitude of the estimated errors, the apparent rate constant measured is approximately that of the rate constant for the transfer of the electron from the tyrosine to the neutral tryptophan radical. [b] An estimated value: DeFelippis, M.; Pippin, P.; Faraggi, M.; Klapper, M. H., unpublished results. [c] Values in parentheses taken from: Prütz, W. A.; Land, E. J.; Sloper, R. W. *J. Chem. Soc., Farad. Trans. 1* **1981**, *77*, 281–292. [d] Where second-order values are given, the concentration of the peptide was varied, and the second-order rate constant determined from the slope of the linearly dependent relationship between the apparent first-order rate constant and the concentration. The abbreviation nd indicates that no concentration dependence was detected, due presumably to the speed of the intramolecular processes. Where there is no entry, only one peptide concentration was used, and we estimate an error of 20–30% associated with the second-order rate constants for the slower reactions in the peptides with the -$(Pro)_n$- spacers. [e] Estimated from the absorbances (A) measured at 2 μs after the pulse (A_0) and after the electron-transfer reaction had reached equilibrium (A_{eq}). Abbreviating 'Trp-X-TyrOH as Trp• and TrpH-X-TyrO• as TyrO• and using the fact that at any wavelength above 360 nm $A_{obsd} = A_{Trp^\bullet} + A_{TyrO^\bullet}$, we can derive the following expression for the equilibrium constant K_{eq}: $K_{eq} = [(\epsilon_{TyrO^\bullet} - \epsilon_{Trp^\bullet})R]/[\epsilon_{TyrO^\bullet}Rd - A_{eq}]$, where $R = \{A_0/d\}/\{[k_1/(k_1 + k_2)]\epsilon_{Trp^\bullet} + [k_2/(k_1 + k_2)]\epsilon_{TyrO^\bullet}\}$, d is the optical path length, ϵ is the extinction coefficient, k_1 is the rate constant for the oxidation of the tryptophan side chain by the azide radical, and k_2 is the rate constant of the tyrosine side chain oxidation. [f] Estimated from data such as those presented in Figure 3. [g] Determined from longer time experiments in which the absorbance decays of the tyrosine and/or tryptophan radical were fit to the kinetic equation expected for a second-order reaction between two identical molecules.

(Table I). With the order of the two amino acids reversed we observe a single broad peak, indicating that the redox potentials of the tyrosine and tryptophan radicals are too close, ≤0.07 V, to be cleanly separated in the scan. We can, however, estimate redox potential differences from the final equilibrium positions attained in the electron-transfer reaction. These equilibrium estimates (Table I) are consistent with the electrochemical results.

A second observation to be drawn from the data of Table I is that the three intramolecular electron rate constants obtained with Tyr–Gly–Trp, Tyr–Glu–Trp, and Tyr–Lys–Trp are approximately the same. With Tyr–Pro–Trp the rate constant drops by a factor of only 3–4. Hence, electron transfer is relatively insensitive to the nature of the central amino acid side chain in a tripeptide. Either electron transfer in tripeptides occurs primarily when tyrosine and tryptophan side chains collide in the normal course of bond rotational motion, or LRET is the predominant redox mechanism, with the charge on the intervening side chain irrelevant in both cases. Were we observing LRET, then the insensitivity to side-chain charge and the slightly slower electron transfer across a central proline might be consistent with electron transfer along the peptide backbone. It is, however, clear that we cannot distinguish between contact and long-range transfer with these results alone.

A third observation is that intramolecular electron transfer in the peptides Tyr-$(Glu)_n$-Trp appears to be almost independent of the number, n, of intervening glutamate residues, with the rate constant dropping only from 2.1×10^4 to 1.5×10^4 s^{-1}. At pH 7 the glutamate side chains carry a negative charge that should keep the tyrosine and tryptophan ends apart. Hence, it may be reasonable to argue for LRET in this series of peptides, but conservatively we still cannot rule out electron transfer due to side-chain collisions.

On the other hand, because of the rigidity of the proline ring, the two ends of a polypropline chain should be held scrupulously apart with the proline peptide bond constrained to two conformations, cis and trans. We know from experiment that the distance between tyrosine and tryptophan separated by an oligoproline spacer increases linearly with the addition of each intervening proline residue, as detected by Förster transfer.[9] Hence, there is little likelihood with the oligoproline spacer that electron transfer

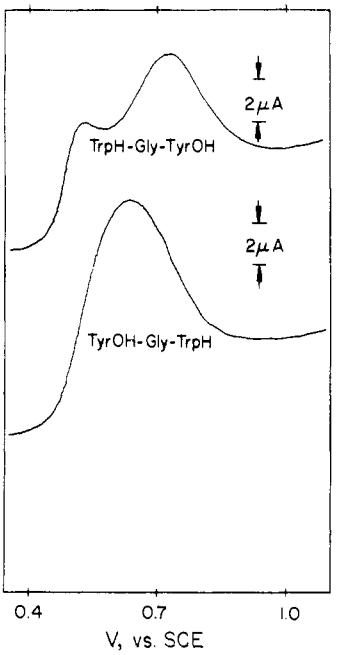

Figure 3. Differential pulse polarography of Trp-Gly-Tyr and Tyr-Gly-Trp. The conditions were similar to those of Figure 1 except for the absence of N_2O and the replacement of sodium azide with sodium chloride. The scan rate was 2 mV/s, and the amplitude 25 mV, peak to peak.

(8) We cannot with these results alone assign absolute redox potentials to the peptide TyrO• and Trp• radicals. From literature reports and our unpublished results free Trp• appears to be a better oxidant than free TyrO•, and we tentatively assign the lower potential peak to tyrosine.

(9) Chiu, H. C.; Bersohn, R. *Biopolymers* **1977**, *16*, 277–288. If all peptide bonds were cis, each proline would add 1.85 Å; if all were trans, 3.12 Å. Chiu and Bersohn estimate each residue adds 2.2 Å to the chain and that the Trp-to-Tyr distance in Trp-$(Pro)_3$-Tyr is 14.0 Å.

is through direct side-chain contact, and we conclude that intramolecular electron transfer in these peptides must be long range. (The indole-to-phenol side chain distance in Trp-$(Pro)_3$-Tyr is 14.0 Å.[9]) What is, therefore, so remarkable is the very slight distance dependence of the k_{app} obtained with these oligoproline peptides. There appears to be little "resistance" to LRET in these peptides, an observation that would be valid provided we were certain that electron transfer occurs as the rate-determining step.

The electron-transfer reactions of eq 3 also include proton transfers. Were a proton transfer reaction rate determining, then the apparent electron-transfer rate constant would not be dependent on the distance between tyrosine and tryptophan. Any interpretation of our kinetics results in terms of the tyrosine-to-tryptophan distance must, therefore, consider the possible importance of proton transfers. Schemes in which an initial fast electron-transfer step is followed by a slow protonation or deprotonation need not be considered as a complicating possibility. Such schemes predict the fast formation and accumulation of either a $TrpH^{\bullet+}$ or $TyrOH^{\bullet+}$ cationic radical intermediate. Either will be spectrally distinct and, hence, should appear as a kinetically significant intermediate. In fact, we have no evidence for any such intermediate as witnessed, for example, by the observed isosbestic point (Figure 2). Of the remaining simple reaction schemes two must still be considered as pathways in which electron transfer might not be rate determining; in the first (eq 4) there is a slow

$$\begin{aligned} &\text{HO-Tyr-X-Trp}^{\bullet} \overset{\text{slow}}{\rightleftharpoons} {}^{-}\text{O-Tyr-X-Trp}^{\bullet} + \text{H}^{+} \\ &\text{TyrO}^{-}\text{-X-Trp}^{\bullet} + \text{H}^{+} \overset{\text{fast}}{\rightleftharpoons} {}^{\bullet}\text{O-Tyr-X-TrpH} \end{aligned} \tag{4}$$

$$\begin{aligned} &\text{TyrOH-X-Trp}^{\bullet} + \text{H}^{+} \overset{\text{slow}}{\underset{l_1}{\overset{k_1}{\rightleftharpoons}}} \text{TyrOH-X-TrpH}^{\bullet+} \\ &\text{TyrOH-X-TrpH}^{\bullet+} \overset{\text{fast}}{\underset{l_2}{\overset{k_2}{\rightleftharpoons}}} \text{TyrO}^{\bullet}\text{-X-TrpH} + \text{H}^{+} \end{aligned} \tag{5}$$

deprotonation to the phenolate ion, which is only then rapidly oxidized by the tryptophan radical; in the second (eq 5) there is a slow protonation of $Trp^{\bullet}$ to form the tryptophan radical cation ($TrpH^{\bullet+}$) that then rapidly oxidizes the tyrosine side chain. Were either the reaction scheme of 4 or 5 valid, then the overall rate of electron transfer would be dependent on the protonation/deprotonation rate. However, we can eliminate both of these possibilities as shown in the Appendix, and the simple schemes that remain are (i) a rate-limiting electron transfer followed by rapid deprotonation of the strong acid radical cation $TyrOH^{\bullet+}$ and rapid protonation of the strong anionic base Trp^{-} and (ii) a single concerted reaction in which protonation/deprotonation and electron transfer occur simultaneously. The one test we made for a concerted mechanism was to look for imidazole catalysis of the intramolecular electron transfer. No catalysis was found. However, this negative result does not by itself exclude a concerted reaction. More complex schemes in which electron transfer is not rate determining can most surely be constructed; however, we invoke Occam's razor and propose that intramolecular electron transfer is rate determining, at least in those peptides with the proline spacer; we plan further experiments to learn more about the operative reaction scheme.

Others have utilized proline as a spacer before us, and so it is worth comparing our results with theirs. Isied and co-workers[11] have measured electron transfer from Os(II) to Co(III) in pulse radiolytically generated binuclear $(NH_3)_5Os^{II}$-L-$Co^{III}(NH_3)_5$ complexes, where L represents an oligoproline-based spacer. Schanze and Sauer[12] have looked at electron transfer between a photoexcited polypyridyl Ru(II) complex donor and a *p*-benzoquinone type electron acceptor also linked by prolines. We present

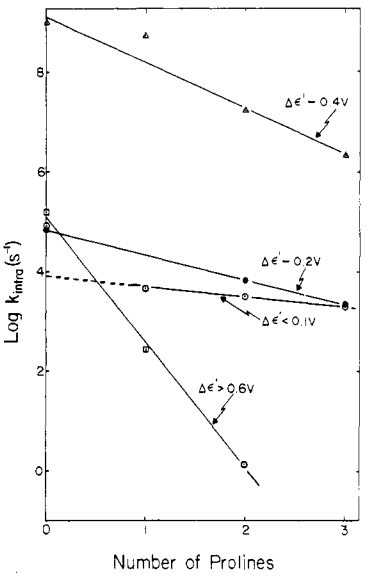

Figure 4. Dependence of the intramolecular electron-transfer rate constant on distance with four different oligoproline systems: ○, this report, Tyr-$(Pro)_n$-Trp; ●, this report, Trp-$(Pro)_n$-Tyr; △, Schanze and Sauer;[12] □, Isied et al.[11]

the comparison of all these results in Figure 4. As is predicted by the most widely used theory of long-range electron transfer,[13] the intramolecular rate constants obtained in the four sets of homologous compounds decrease exponentially (perhaps) with increasing distance. That the slopes of the four curves differ even though -(proline)$_n$- is the intervening chain in each case is of some interest. While one should be careful in comparing disparate data,[14] there does appear to be a correlation between the estimated redox potential differences of the electron donor/acceptor pairs and the slopes of the semilogarithmic curves; the greater the redox potential difference, the more negative the slope. One result of this correlation is a crossover; over longer distances electron transfer between tyrosine and tyrptophan can be faster when the redox potential difference is smaller, with the opposite seen over smaller distances.

LRET has been described as a multicomponent process with an electron-transfer rate constant, k_{et}, that can be decomposed into

$$k_{et} = k_{el}\nu k_{nuc} \tag{6}$$

where k_{el} is the electronic transmission coefficient, ν a nuclear vibrational frequency, and k_{nuc} a nuclear factor. While it has long been presumed that $k_{el}\nu$ has an exponential dependence on the distance between redox centers:

$$k_{el}\nu = A \exp[-\beta(r - r_0)] \tag{7}$$

there may be reason to argue that k_{nuc} also has an exponential distance dependence.[15] According to theory

$$k_{nuc} = \exp\left[-\frac{(\lambda + \Delta G^{\circ})^2}{4\lambda RT}\right] \tag{8}$$

(10) Rodiguin, N. M.; Rodiguina, E. N. *Consecutive Chemical Reactions: Mathematical Analysis and Development*; van Nostrand: Princeton, NJ, 1964; pp 42–43.

(11) Isied, S. S.; Vassilian, A.; Magnuson, R. H.; Schwarz, H. A. *J. Am. Chem. Soc.* **1985**, *107*, 7432–7438.

(12) Schanze, K. S.; Sauer, K. *J. Am. Chem. Soc.* **1988**, *110*, 1180–1186.

(13) Marcus, R. A.; Sutin, N. *Biochem. Biophys. Acta* **1985**, *811*, 265–322.

(14) Although we compare the Figure 4 data with such facility, there are important experimental differences. Schanze and Sauer[12] studied a photoinduced electron transfer with the donor/acceptor molecules dissolved in methylene chloride, while our results and those of Isied et al.[11] were obtained with pulse radiolysis and aqueous solutions. Not only is there a dielectric constant difference, but the proline peptide bond cis–trans equilibrium is not shifted as far to the trans conformation in apolar solvents. Without a net charge separation the electron-transfer standard free energy should not change with chain length in either of the two solvents. But the "ruler" distance between redox centers may be shorter in methylene chloride than in water.

(15) (a) Oevering, H.; Paddon-Row, M. N.; Heppener, M.; Oliver, A. M.; Cotsaris, E.; Verhoeven, J. W.; Hush, N. S. *J. Am. Chem. Soc.* **1987**, *109*, 3258–3269. (b) Isied, S. S.; Vassilian, A.; Wishart, J. F.; Creutz, C.; Schwarz, H. A.; Sutin, N. *J. Am. Chem. Soc.* **1988**, *110*, 635–637.

with $\Delta G°$ the standard free energy for the overall reaction and λ a nuclear reorganization free energy. In the reactions summarized in Figure 4 there is no charge separation and $\Delta G°$ should be distance independent. Hence, the dependence of the slopes (Figure 4) on the redox potential difference suggests that β of eq 7 and/or λ of eq 8 is dependent on the electron-transfer net free energy, if we assume that these equations are appropriate. We know of no other experimental observation that is similar to our own, nor do we have an explanation based on eq 6–8 for a free energy correlation in the ln k_{app} versus distance plot. This apparent correlation could be merely fortuitous, a point we shall continue to investigate.

The now frequent observation of long-range electron transfer in proteins suggests that close physical proximity between redox centers is not necessary for biologically significant electron transfer. Our results, although obtained for a process not (yet) found in nature, have additional physiological implications. First, biological electron transfers often involve donor/acceptor pairs with small redox potential differences, e.g., electron transfer along the cytochrome paths of respiration or photosynthesis. With the tyrOH/trpH pair we see a small potential energy difference between donor and acceptor and no precipitous fall of the electron-transfer rate with increasing distance; LRET over long distances is possible in proteins even when the potential energy difference is small. Second, there is evidence to suggest that electron transfer within proteins may be directed toward specific target sites or along particular pathways.[16] Such specificity, were it real, would imply either structural kinetic control that directs LRET along a preferred path and/or thermodynamic control in the form of potential energy traps constructed at appropriate sites. Either mechanism could be an evolutionary tool to enhance electron transfer between "biologically correct" groups not in direct physical contact.

Materials and Methods

All peptides purchased from Bachem Bioscience (Philadelphia, PA) were used with no further purification. Water was obtained from a Millipore Milli-Q apparatus. All other chemicals were used as purchased from standard sources.

The electron-transfer reactions were initiated with radicals generated by introduction into aqueous solutions of a 100–200-ns high-energy electron pulse (ca. 4 MeV with the two Varian linear accelerators located at the Ohio State University Department of Chemistry[17a] and the Hebrew University, Jerusalem[17b]). The primary radicals ($e_{aq}^{\bullet-}$, $OH^{\bullet}$) formed during the pulse were converted to $N_3^{\bullet}$ in the reactions of eq 1 when the solutions contained saturating N_2O and 0.05–0.1 mM azide. The solutions also contained 5 mM phosphate buffer, pH 7.0. the electron-beam current was attenuated with aluminum filters so that the total azide radical concentration was ≤ 1 μM. These low concentrations were used so as to minimize the second-order decays of tyrosine and tryptophan radicals. The peptides were present at ~ 1 mM, except when a k_{app} concentration dependence was sought, and they were varied over the range 0.05–1 mM. All equilibration reactions, monitored at 510 and/or 410 nm, were functionally first order, and all radical decay reactions functionally second order. The rate constants were extracted from all the results by nonlinear least-squares analysis of the absorbance data.

We performed the differential pulse polarography experiments using a Model 173D potentiostat/galvanostat, a Model 179 digital Coulometer, a Model 175 universal programmer, and a Model 174A polarographic analyzer, all from Princeton Applied Research (Princeton, NJ). The experiments were conducted in a three-electrode glass cell (Metrohm) with a glassy carbon working electrode of 0.2-cm^2 exposed area. All solutions contained 0.1 M KCl and 5 mM phosphate buffer, pH 7.0, and were degassed with argon by bubbling for 1 h.

Appendix

The initial phenolate intermediate scheme of eq 4 is ruled out by the following argument. The pK_a of the tyrosine phenol is close to 10. Protonation of the phenolate anion can occur no faster than dictated by diffusion; i.e., the rate constant for this reaction can be no higher than a generous 5×10^{10} M^{-1} s^{-1}. If we, therefore, make the reasonable assumption that the phenol/phenolate equilibrium is an elementary process, then

$$k_{\text{deprotonation}} = K_a k_{\text{protonation}} \approx (10^{-10}\ \text{M})(5 \times 10^{10}\ \text{M}^{-1}\ \text{s}^{-1}) = 5\ \text{s}^{-1}$$

The fastest possible rate constant for the first step in the scheme of eq 4 is too slow for even the slowest measured LRET constant, 2000 s^{-1}.

To rule out the scheme of eq 5, the postulated tryptophan radical cation intermediate, we need a more prolix argument. Since the experimental hydronium ion concentration is effectively constant, both reactions of this scheme are pseudo-first-order, and the complete solution for this scheme is already available in the literature.[10] The time dependence of the overall reaction is described by two apparent rate constants, the negative roots of the equation

$$\alpha^2 + \alpha[k_1(H^+) + l_2(H^+) + k_2 + l_1] + [l_1 l_2 + k_1 k_2](H^+) + k_1 l_2 (H^+)^2 = 0 \quad \text{(A1)}$$

We can simplify (A1) as follows. First, the pK_a of trpH$^{\bullet+}$ is approximately 4.3;[5] hence

$$K_a = l_1/k_1 \approx 5 \times 10^{-5} \Rightarrow l_1 \approx 5 \times 10^{-5} k_1$$

Second, the overall equilibrium constant for the electron-transfer process in the TyrOH-X-TrpH peptides is approximately 10 (obtained from the estimated redox potential difference of 60 mV in Table I); hence

$$K_{eq} = k_1 k_2 / l_1 l_2 \approx 10$$

Substituting these two relationships and the fact that the solution pH was 7 results in

$$\alpha^2 + \alpha(5.0 \times 10^{-5} k_1 + k_2) + 1.1 k_1 k_2 \times 10^{-7} \approx 0 \quad \text{(A2)}$$

From the solution of (A2), we find that consistent with the experimental observation of a single apparent rate constant, there can be only one significant root, and since $k_{app} = -\alpha$ we obtain the approximation

$$k_{app} \approx 5.0 \times 10^{-5} k_1 + k_2 \quad \text{(A3)}$$

From our experiments with the TyrOH-X-TrpH peptide series we know that k_{app} must vary between 2000 and 7000 s^{-1}. Using the larger of these two values, we can estimate the magnitudes of the two forward rate constants k_1 and k_2 at any given value of the ratio $10^{-7} k_1/k_2$. The factor of 10^{-7} is necessary since the first protonation step as written is pseudo-first-order at fixed pH with an apparent rate constant of $k_1(H^+)$, and the experimental pH is 7.0. For the protonation step to be rate determining $k_1(H^+)/k_2$ must be <1. When this ratio is varied from 1 to 0.0001, the calculated value of $k_1(H^+)$ varies from 14 to 0.7 s^{-1}. The computed value of $k_1(H^+)$ is even lower when we set k_{app} to 2000 s^{-1}. Clearly, the rate-determining step cannot be as slow as the computed value of $k_1(H^+)$ since the actual k_{app} is $>10^3$ s^{-1}. Hence, there is a contradiction that indicates an inconsistency between the eq 5 scheme and our experimental results.

(16) Steiner, J. P.; Faraggi, M.; Klapper, M. H.; Dorfman, L. M. *Biochemistry* **1985**, *24*, 2139–2146. Liang, N.; Pielak, G. J.; Mauk, A. G.; Smith, M.; Hoffman, B. M. *Proc. Natl. Acad. Sci., U.S.A.* **1987**, *84*, 1249–1252.

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