Electron Transfer

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Influence of Amino Acid Side Chains on Long-Distance Electron Transfer in Peptides: Electron Hopping via "Stepping Stones"**

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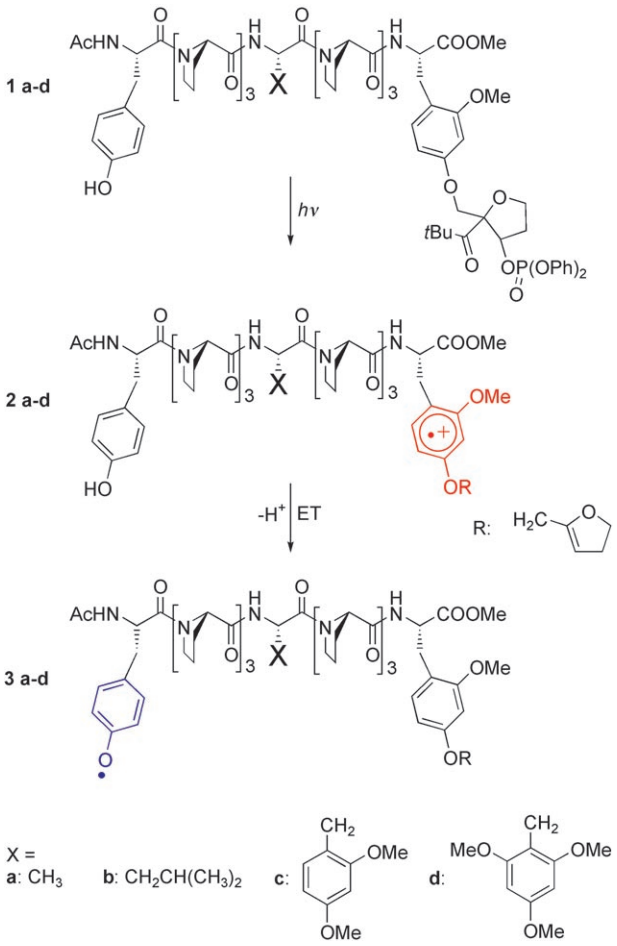
Dedicated to Professor Andreas Pfaltz on the occasion of his 60th birthday

Electron transfer (ET) processes through proteins play an important role in many biological reactions. Seminal work by Gray and Winkler on Ru-modified proteins, such as cytochromes and azurine, has shown that long-distance ET can occur over more than 20 Å.[1,2] Beratan and Onuchic developed a "pathway model",[3] which explains ET through these proteins by single-step superexchange (tunneling) reactions. The model comprises a "family of pathways" involving ET through σ bonds, through hydrogen bridges, and through space.[4] In contrast, Stubbe, Nocera, et al. explained long-distance ET through ribonucleotide reductase by a multistep hopping mechanism, where electrons hop between aromatic side chains of amino acids.[5,6] These amino acids, which carry the charge for a short time, act as "stepping stones" (relay stations) for long-distance electron transport from the donor to the acceptor.[7] For consecutive reactions of this kind,[8] oxidized donor, acceptor, and relay amino acids should in principle be present at the same time during the ET process. We have now developed a model peptide, where the simultaneous existence of all the oxidized intermediates (oxidized amino acid side chains) could be proven spectroscopically for the first time.

To test the influence of aromatic amino acids on long-distance electron transfer (ET) in peptides we synthesized model systems **1a–d**,[9] in which three amino acids are separated from each other by triproline sequences (Scheme 1). A positive charge was selectively injected into the aromatic side chain of the C-terminal amino acid (**1**→**2**), which then served as the electron acceptor. A tyrosine residue at the N terminus, about 20 Å away,[10] functioned as the electron donor. Halfway between the donor and the acceptor we introduced an amino acid with either an aliphatic or an aromatic side chain X (Scheme 1).

The function of the charge injection system at the C-terminal amino acid of molecules **1a–d** is depicted in Scheme 2. Photocleavage of the ketone leads to radical **4**, which undergoes a heterolytic β fragmentation (**4**→**5**) to give



Scheme 1. Injection of a positive charge into the C-terminal aromatic amino acid of an oligopeptide and subsequent electron transfer from the N-terminal tyrosine residue.

a radical cation **5**,[11] which then selectively oxidizes the attached aromatic ring (**5**→**2**). The transient absorption spectrum of the electron acceptor in **2** shows a maximum at 450 nm (Figure 1).

The signal of **2** vanishes as a consequence of electron transfer from tyrosine (**2**→**3**), which deprotonates upon oxidation and yields a tyrosyl radical (Figure 1). The acceptor radical cations **2a–d** were generated by laser flash photolysis (LFP) of the precursors **1a–d**,[12] and the transient absorption spectra were measured 40 ns after the laser flash.[13] To check whether *intermolecular* ET already competes with *intramo-*

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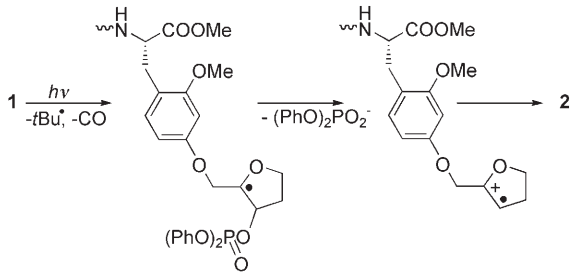


Scheme 2. Generation of a radical cation by laser flash photolysis (LFP).

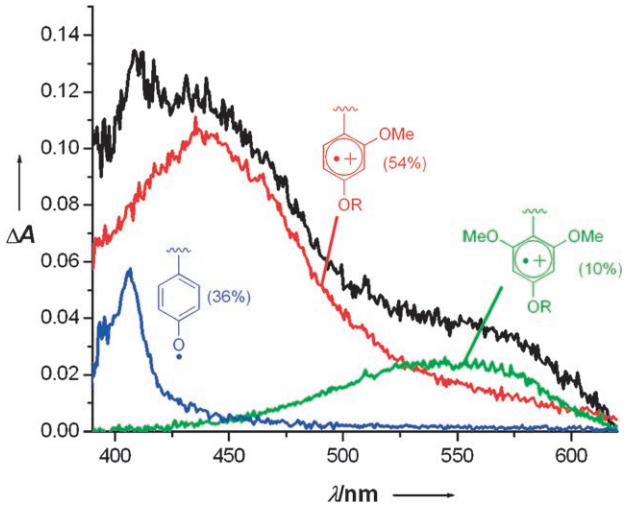
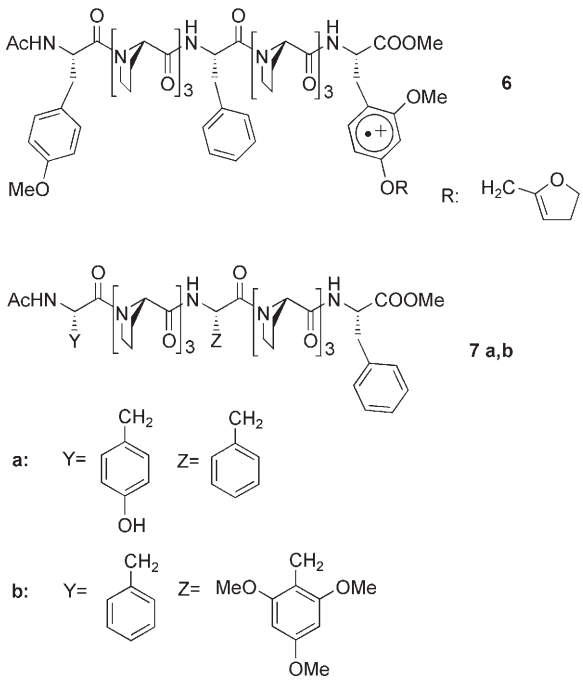


Figure 1. Transient absorption spectrum of oligopeptide **1d** 40 ns after the laser flash (black line), tyrosyl radical (blue),[14] the 2,4,6-trimethoxyphenylalanine radical cation (green),[17] and the dialkoxyphenylalanine radical cation **6** (red). The colored spectra are fitted to estimate the amounts of transients generated by the LFP of **1d**. The overall amount of transients is set to 100%.



Scheme 3. Peptides used for control experiments to estimate the importance of intermolecular electron transfer.

Table 1: Yield of tyrosyl radicals within 40 ns after LFP of **1a–d**.[a]

Molecule	Tyrosyl radical (detected) [%][b]	Tyrosyl radical (corrected) [%][c]
6 + **7a**	6.1	–
1a	6.9	≦1
1b	6.1	≦1
1c	27	21
1d	36	30

[a] Relative error for all values: 10%. The sum of the oxidized aromatic side chains is set to 100%. The amount of tyrosyl radical formed by intermolecular reaction of **6** and **7b** is given for comparison. [b] Corrected on the basis of the extinction coefficients for the tyrosyl radical ($3000\,M^{-1}cm^{-1}$),[14] the 1,3-dimethoxybenzene radical cation ($4000\,M^{-1}cm^{-1}$),[15] and the 1,3,5-trimethoxybenzene radical cation ($4000\,M^{-1}cm^{-1}$).[15,16] [c] Corrected by the amount of tyrosine oxidized by intermolecular ET, as estimated from the reaction of **6** and **7a**.

lecular ET during this time, the tyrosine-free radical cation **6** was generated (Scheme 3).

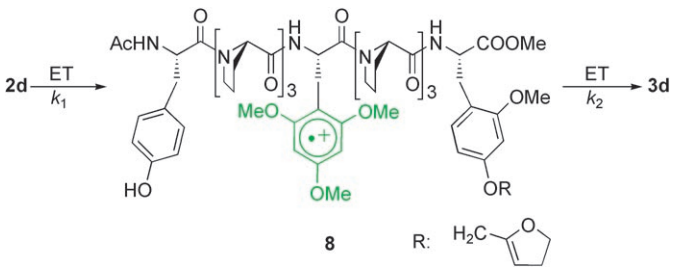
Experiments with mixtures of **6** and oligopeptide **7a**, which contains a tyrosine residue but no injector, showed that a small amount of tyrosyl radicals (6.1% of the overall transient species) was already formed by intermolecular ET 40 ns after the laser flash (Table 1).

Thus, only yields of tyrosyl radicals above 6% represent intramolecular ET processes (**2**→**3**) in the LFP experiments with peptides **1a–d**. With alanine and leucine as central amino acids (**1a** and **1b**, respectively), between 6 and 7% of tyrosyl radicals were formed 40 ns after irradiation (Table 1). Therefore, 1% of the tyrosine at most was oxidized by intramolecular ET. However, when the aliphatic side chains X were exchanged by aromatic side chains (**1c** and **1d**) we detected tyrosyl radicals in yields of 27 and 36%, respectively (Table 1). This finding demonstrates that the aromatic side chains X increase the intramolecular ET rates (**2**→**3**) by a factor of at least 20 to 30.

Whether this rate enhancement is caused by an increase in the electronic coupling between the donor and acceptor according to a single-step superexchange mechanism, or whether a mechanistic change to a sequential hopping mechanism speeds up the ET rates, could be elucidated in experiments with peptide **1d**.

The transient absorption spectrum of **1d**, taken 40 ns after the laser flash (Figure 1), demonstrates the simultaneous existence of not only remaining electron acceptor (**2d**, 54%) and oxidized electron donor (**3d**, 36%), but also of the oxidized amino acid (**8**, 10%, $\lambda_{max} = 550$ nm) that is located halfway between the electron donor and electron acceptor.[17] The intermediate radical cation **8** is generated by intramolecular ET, since intermolecular ET from the central trimethoxyphenylalanine to an electron acceptor, as in **6**, could not be detected in experiments with peptide **7b**.

Thus, long-distance ET from the donor to the acceptor (**2d**→**3d**) uses the central aromatic amino acid as a "stepping stone", and ET takes place by a two-step hopping mechanism (Scheme 4). The sequential mechanism is favored, since the redox potential of the "relay amino acid" is similar to that of the C-terminal electron acceptor.[18] Introduction of the "stepping stone" leads to a 20- to 30-fold increase in the ET rate.



Scheme 4. Electron transfer in **2d** occurs via intermediate **8**.

From the kinetic expression for a consecutive reaction mechanism[8] and the yields of the oxidized N-terminal, C-terminal, and "relay amino acids", one can calculate that the first ET step (k_1) is about five- to six-times slower then the second ET step (k_2). This result is in accord with the differences in the redox potentials of the involved aromatic side chains.[18] The overall intramolecular ET rate is on the order of $10^6\ s^{-1}$ because this reaction competes successfully with intermolecular ET, which has a rate coefficient of $3 \times 10^8\ s^{-1}$,[17] at the 5 mM peptide concentrations used in the LFP experiments.

In conclusion, we have shown that ET in peptides **2c** and **2d** occurs by a two-step hopping process, with the aromatic side chains used as "stepping stones". This hopping of the electrons over 20 Å leads to a 20- to 30-fold increase in the reaction rate compared to superexchange ET in petides **2a** and **2b**.

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[1] H. B. Gray, J. R. Winkler, *Q. Rev. Biophys.* **2003**, *36*, 341.
[2] H. B. Gray, J. R. Winkler, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3534.
[3] D. N. Beratan, J. N. Onuchic, *Photosynth. Res.* **1989**, *22*, 173.
[4] Refinement of this model, by taking protein dynamics into account, enabled good agreement with the experimental data (Refs. [1,2]) to be achieved: T. R. Prytkova, I. V. Kurnikov, D. N. Beratan, *Science* **2007**, *315*, 622; S. S. Skourtis, I. A. Balabin, T. Kawatsu, D. N. Beratan, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3552; for further recent contributions on the influence of backbone conformations on electronic coupling, see E. W. Schlag, S.-Y. Sheu, D.-Y. Yang, H. L. Selzle, S. H. Lin, *Angew. Chem.* **2007**, *119*, 3258; *Angew. Chem. Int. Ed.* **2007**, *46*, 3196.
[5] J. Stubbe, *Curr. Opin. Chem. Biol.* **2003**, *7*, 183.
[6] J. Stubbe, D. G. Nocera, C. S. Yee, C. Y. Chang, *Chem. Rev.* **2003**, *103*, 2167.
[7] For further experiments in which a charge hopping through side chains or peptide bonds are discussed, see K. Yanagisawa, T. Morita, S. Kimura, *J. Am. Chem. Soc.* **2004**, *126*, 12780; T. Carell, L. T. Burgdorf, L. M. Kundu, M. Cichon, *Curr. Opin. Chem. Biol.* **2001**, *5*, 491; C. Aubert, M. H. Vos, P. Mathis, A. P. M. Eker, K. Brettel, *Nature* **2000**, *405*, 586; E. W. Schlag, D.-Y. Yang, S.-Y. Sheu, H. L. Selzle, S. H. Lin, P. M. Rentzepis, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9849; C. C. Page, C. C. Moser, X. X. Chen, P. L. Dutton, *Nature* **1999**, *402*, 47.
[8] R. Huisgen in *Methoden der Organischen Chemie (Houben-Weyl)*, *Vol. 3* (Ed.: E. Müller), Thieme, Stuttgart, **1955**, pp. 139.
[9] The enantioselective syntheses of the new aromatic amino acids follow the Erlenmeyer-azlactone synthesis and are reported in: M. Cordes, O. Jacques, A. Köttgen, C. Jasper, H. Boudebous, B. Giese, *Adv. Synth. Catal.*, in press.
[10] The CD spectra of compounds **1a–d**, **6**, and **7** show a negative band around 206 nm and a positive band around 229 nm, which are characteristic of a PPII helix. The helices do not melt upon heating to 80 °C. The PPII helix has a repeat unit of three proline molecules with an axial translation of 3.2 Å per residue, see, for example: S. Kakinoki, Y. Hirano, M. Oka, *Polym. Bull.* **2005**, *53*, 109. In an ideal PPII helix, the side chains of the donor, acceptor, and relay amino acids in compounds **1a–d** will be at an angle of 120° to each other.
[11] We have already used and studied a similar system for the injection of a positive charge into the heterocyclic bases of DNA: E. Meggers, M. E. Michel-Beyerle, B. Giese, *J. Am. Chem. Soc.* **1998**, *120*, 12950; E. Meggers, D. Kusch, M. Spichty, U. Wille, B. Giese, *Angew. Chem.* **1998**, *110*, 473; *Angew. Chem. Int. Ed.* **1998**, *37*, 460.
[12] Degassed solutions of **1a–d**, **6**, and **7** (5 mM in acetonitrile/water 3:1) were irradiated at a wavelength of 308 nm in a quartz cell of 4.5 cm path length with a Lambda-Physik COMPex 205 laser (pulse width: 25 ns, 100–150 mJ per pulse) at RT. An iStar 720 ICCD camera (Andor) was used for the detection of transient absorption spectra 40 ns after irradiation. The signals of five flashes were accumulated to improve the signal/noise ratios.
[13] By competition experiments we determined the rate for the β fragmentation (**4**→**5**) to be $2 \times 10^8\ s^{-1}$. Therefore, UV detection of the intermediates before 40 ns is not useful.
[14] R. H. Schuler, P. Neta, H. Zemel, R. W. Fessenden, *J. Am. Chem. Soc.* **1976**, *98*, 3825.
[15] P. O'Neill, S. Steenken, D. Schulte-Frohlinde, *J. Phys. Chem.* **1975**, *79*, 2773.
[16] N. Ichinose, T. Tanaka, S. Kawanishi, T. Suzuki, *J. Phys. Chem. A* **1999**, *103*, 7923.
[17] B. Giese, M. Napp, O. Jacques, H. Boudebous, A. M. Taylor, J. Wirz, *Angew. Chem.* **2005**, *117*, 4141; *Angew. Chem. Int. Ed.* **2005**, *44*, 4073.
[18] The oxidation potentials of the aromatic amino acids were measured in acetonitrile by differential pulse voltammetry with ferrocene as an internal standard (working electrode: glassy carbon, (TBA)PF_6 as electrolyte (TBA = tetrabutylammonium), scan rate $100\ mV\ s^{-1}$): *N*-Ac-2,4-dimethoxyphenylalanine methyl ester 0.95 V; *N*-Ac-2,4,6-trimethoxyphenylalanine methyl ester 0.94 V; *N*-Ac-tyrosine methyl ester 0.85 V (versus the ferrocene/ferrocinium (Fc/Fc^+) couple).

