Electron Transfer in Peptides

Multistep Electron Transfer in Oligopeptides: Direct Observation of Radical Cation Intermediates**

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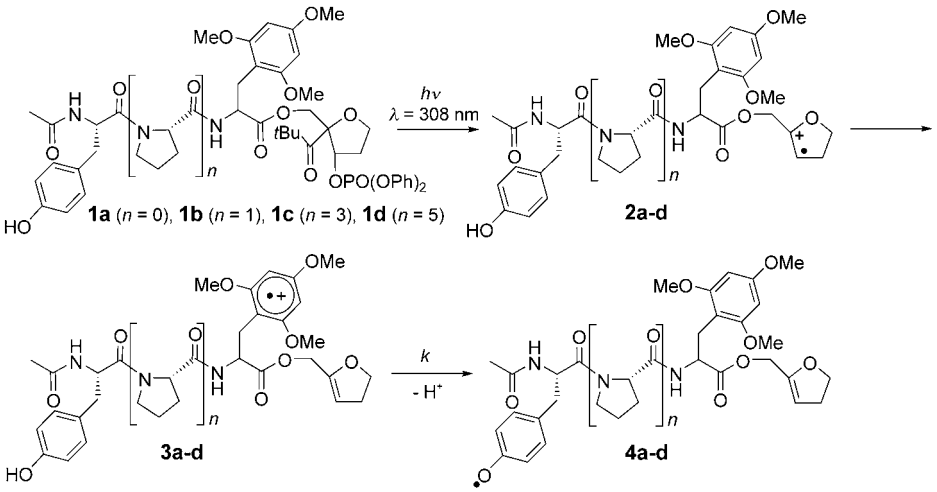
For the transport of electrons or electron holes over long distances, nature often uses DNA double strands or proteins. With DNA, experiments[1] and calculations[2] have clearly shown that these processes can occur by a hopping mechanism, where the charge transport takes place in a multistep reaction.[3] Purines act as carriers of positive charge[4] ("electron-hole transport"), and pyrimidines are carriers of negative charge[5] ("excess-electron transport"). A similar hopping model was also applied to explain long-distance charge transport through proteins.[6] Hopping is especially favored if the amino acids carry side chains that can be easily oxidized (tyrosine, tryptophan, and histidine). For instance, a multistep electron-hole transport cascade involving three tryptophan units was proposed for the photoactivation process in the enzyme photolyase.[6a] Presumably, not only tryptophan but also tyrosine and histidine are involved in the mechanism of radical initiation in ribonucleotide reductase, where an electron hole is transported over a distance of 35 Å.[6e] In these systems, transient intermediates occurring in the multistep charge transport have not yet been detected, and it is not clear whether the electron transfer is best described as proceeding through space or through the peptide bonds.[7]

In order to examine electron-hole transport in peptides, we synthesized the series of model systems **1a–d** bearing three different redox sites, that is, a modified tetrahydrofuran as a charge-injection system,[8] 2,4,6-trimethoxyphenylalanine[9] as an intermediate charge carrier, and tyrosine as a final electron donor. An oligoproline spacer of variable length ($n = 0$, 1, 3, 5) was chosen to separate the tyrosine residue from the 2,4,6-trimethoxyphenylalanine moiety. As is known from related studies of electron-hole transfer in DNA, irradiation of the pivaloylated tetrahydrofuran unit in compounds like **1a–d** will generate the dihydrofuranosyl radical cations, **2a–d**, through Norrish α cleavage and subsequent β elimination of the phosphate leaving group.[10] These radical cations have



Scheme 1. Mechanism for the photolysis of **1a–d** and the subsequent electron-transfer reactions.

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strong oxidizing properties[11] and can oxidize the neighboring 2,4,6-trimethoxyphenylalanine side chain (Scheme 1).[12]

Compounds **1a–d** were dissolved in acetonitrile/water (3:1) and irradiated with 308-nm pulses from a XeCl excimer laser,[13] which allowed selective excitation of the pivaloyl chromophore. The transient absorption spectra observed after a delay of 50 ns with respect to the laser pulse indicated the presence of radical cations **3a–d** with an absorption maximum at 550 nm (Figure 1a shows the spectrum for system **1c** as a typical example).[14,15] This signal decayed

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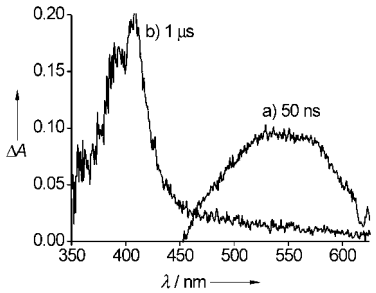

Figure 1. Transient absorption spectra observed after a delay of a) 50 ns and b) 1 μs with respect to the irradiation of **1c** in degassed acetonitrile/water (3:1) at a concentration of 8 mM.

within 1 μs and was replaced by the characteristic signal of the tyrosyl radical with a maximum absorbance at 410 nm (Figure 1 b).[16]

Kinetic traces of the absorbance decay at 550 nm and of the growth observed at 410 nm obeyed the first-order rate law well, and the rate constants determined by least-squares fitting at the two wavelengths were the same within the limits of error. A typical example is shown in Figure 2.

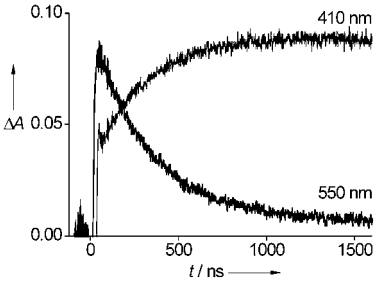

Figure 2. Kinetic traces of the transient absorptions at 410 and 550 nm for **1c** (7 mM) in degassed acetonitrile/water (3:1). The decay rate constant at 550 nm is $3.1(\pm0.2)\times10^6\ s^{-1}$ and the absorbance growth constant at 410 nm is $3.5(\pm0.3)\times10^6\ s^{-1}$.

However, the rate constants observed with model peptides **1a–d** increased linearly with the concentration of these compounds. This pointed to competition of intra- and intermolecular electron transfer. The slopes of these plots correspond to the intermolecular rate contributions k_{inter}, and the values so obtained ($k_{inter}\approx4\times10^8\ M^{-1}s^{-1}$) agree well with the rate constants of diffusion expected for compounds of this size. The intramolecular contributions, namely the rate constants k_{intra}, for electron transfer from the tyrosine side chain to the 2,4,6-trimethoxyphenylalanine radical cation moiety (**3a–d**→**4a–d**) were determined from the intercepts (Table 1).[17]

The rate constants k_{intra} (Table 1) decrease by a factor of only about two when the spacer between the aromatic redox centers is elongated by addition of two proline units (compare **1b** with **1c** and **1c** with **1d**). For an electron transfer through the peptide bonds one would expect a rate decrease of about 500 for two additional proline units.[18] This indicates that the electron transfer does not proceed by a through-bond mechanism. We attribute the small influence of additional proline spacers upon the k_{intra} value to the conformational flexibility of the aromatic amino acids.[19] The rate does increase considerably when the proline spacer is absent, as in **1a**, where the aromatic side chains are in close contact with each other.

Table 1: Rate constants for intramolecular (k_{intra}) and intermolecular (k_{inter}) electron transfer, determined from the intercepts and slopes of rate-versus-concentration plots. The number of proline units between the aromatic side chains is given by n, and σ_l is the length of the through-bond pathway, based on the number of covalent bonds between the aromatic redox centers.[6f]

	n	k_{intra} [s^{-1}]	k_{inter} [$M^{-1}s^{-1}$]	σ_l [Å]
1a	0	$8.6(\pm0.6)\times10^6$	$\leq5\times10^8$	9.8
1b	1	$1.1(\pm0.1)\times10^6$	$4.3(\pm0.4)\times10^8$	14.0
1c	3	$5.1(\pm0.3)\times10^5$	$3.0(\pm0.1)\times10^8$	22.4
1d	5	$2.8(\pm0.3)\times10^5$	$3.2(\pm0.1)\times10^8$	30.8

The radical cation of 1,3,5-trimethoxybenzene[12] should not be capable of oxidizing phenol to the phenol radical cation (oxidation potential $E_{ox}(PhOH/PhOH^{\bullet+})=1.50$ V versus a NHE).[20] But oxidation of phenol to the phenoxy radical in aqueous solution (pH 7) has a much lower potential of $E_{ox}(PhOH/PhO^{\bullet})=0.86$ V versus a NHE.[21] Thus, oxidation of tyrosine in the systems **3a–d** can easily occur, if the electron transfer is coupled with proton transfer to the solvent. We measured a kinetic deuterium isotope effect k_H/k_D of 1.8(±0.2).[22] This value is in good agreement with the isotope effect of 2.0–2.5 for the oxidation of tyrosine by a ruthenium–tris(bipyridine) complex in aqueous media, where a proton-coupled electron transfer occurs.[23]

In summary, electron-hole transport in peptide radical cations **3a–d** occurs by a hopping mechanism between the aromatic side chains of the amino acids, which is similar to the long-distance hole transport through DNA.[1–4]

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[15] The transient absorption spectrum recorded after oxidation of *N*-acetyl-2,4,6-trimethoxyphenylalanine methyl ester (**5**) by photosensitized electron transfer with 1,4-dicyanonaphthalene as a sensitizer and biphenyl as a codonor is similar to the spectrum shown in Figure 1a (see the Supporting Information). Furthermore, a compound with the pivaloylated tetrahydrofuran unit directly connected to *N*-acetyl-2,4,6-trimethoxyphenylalanine was synthesized (**6**). Laser flash photolysis of this compound again gave a spectrum that was practically identical to the one shown in Figure 1a (see the Supporting Information).

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[18] By addition of two proline units, the length of the through-bond pathway (σ_l, 1.4 Å times the number of covalent bonds in the effective path) is increased by 8.4 Å. With a distance-decay constant of $\beta = 0.73\ Å^{-1}$, the k_{intra} value is expected to drop by a factor of about 500: D. R. Casimiro, L. L. Wong, J. L. Colon, T. E. Zewert, J. H. Richard, I.-J. Chang, J. R. Winkler, H. B. Gray, *J. Am. Chem. Soc.* **1993**, *115*, 1485.

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