Electron Transfer

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Electron Transfer in Peptides with Cysteine and Methionine as Relay Amino Acids**

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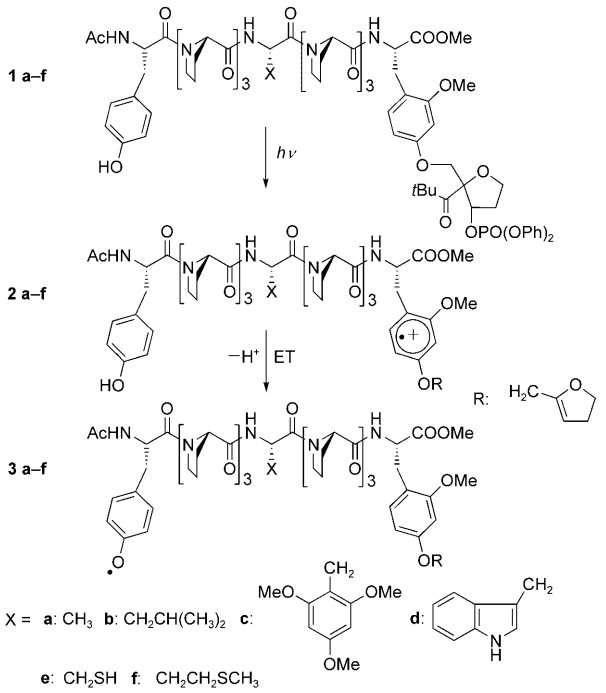
Recently, we developed a peptide system **1** which allows the detection of a multistep hopping process in electron-transfer (ET) reactions through peptides.[1] As the rate for a single-step ET reaction between an electron donor (D) and an electron acceptor (A) decreases exponentially with the distance,[2] long-range ET is fast only if a multistep hopping process occurs.[3] According to this mechanism, the overall distance between D and A is split into shorter and, therefore, faster ET steps. Relay amino acids act as stepping stones for these multistep reactions by acting as intermediate charge carriers.[1] Until now, only aromatic amino acids such as tyrosine[4] and tryptophan[5,6] have been discussed as relay amino acids. We now show that the aliphatic amino acids cysteine and methionine can also function as relay amino acids in ET through peptides.

Our peptide system **1** contains tyrosine at the N-terminal end as the electron donor (D), a dialkoxyphenylalanine at the C-terminal end as a precursor for the electron acceptor (A), and a possible relay amino acid with a side chain X half-way between D and A. These functional amino acids are separated from each other by triproline sequences that induce formation of a rigid PPII helix with a distance of about 20 Å between D and A.[1] Laser photolysis of **1** generates the active peptide **2**, and the ET efficiency is determined from the concentration of the tyrosyl radical (**3**) generated through an intramolecular reaction 40 ns after the laser flash (Scheme 1).[7] The percentage values cited here for the tyrosyl radical are based on this concentration.

Last year we observed that the aliphatic amino acids alanine and homoleucine cannot act as stepping stones, whereas trimethoxyphenylalanine is a perfect relay amino acid that forms a radical cation as a short-lived intermediate during the ET process.[1] From the concentrations of the tyrosyl radicals **3a–c** formed 40 ns after irradiation of peptides **1a–c** (Table 1), it could be deduced that a two-step ET over 20 Å is about 30 times faster than a single-step reaction (Table 1, entries 1–3).[1]

As tryptophan has a lower redox potential (1.0 V versus NHE)[8] than the relay amino acid trimethoxyphenylalanine (1.3 V versus NHE),[9] it should act as a relay amino acid, as has been described by the research groups of Brettel,[5] Stubbe,[4] and Gray.[6] We therefore introduced tryptophan into our peptide (**1d**) and triggered ET by a laser flash. As the low concentration of the formed tyrosyl radical **3d** demonstrates (Table 1, entry 4), the aromatic amino acid tryptophan seems to be nearly as inefficient as a relay amino acid as the aliphatic amino acids alanine and homoleucine (Table 1, entries 1 and 2). However, in contrast to the cases with aliphatic amino acids, new signals appeared in the tryptophan experiment.[10] Comparison with the UV spectra reported by



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

Table 1: Concentration of tyrosyl radicals **3a–f** generated by intramolecular ET after 40 ns.[7]

Entry	Molecule	Central amino acid	Tyrosyl radical [%][a]
1	**1a**	alanine	≤1
2	**1b**	homoleucine	≤1
3	**1c**	trimethoxyphenylalanine	30
4	**1d**	tryptophan	≤2[b]
5	**1e**	cysteine	15[c]
6	**1f**	methionine	20[c]

[a] Percentage based on the amount of radicals and radical ions. [b] About 30% of oxidized tryptophan intermediates were observed. [c] The intermediate sulfur-containing radical were not detected.

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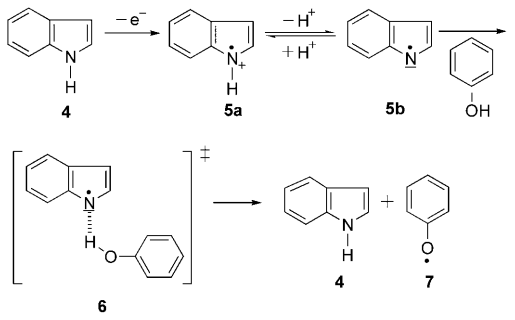
Jovanovic and Simic[11] showed that the new signals belong to the oxidized tryptophan radical cation **5a** and its deprotonated radical **5b**. In the presence of a pH 7.0 buffer (5 mM triethylammonium acetate), radical **5b** was the major intermediate (**5a**:**5b** = 1:10) 40 ns after the laser flash. This finding was surprising because experiments by Brettel and co-workers[5] demonstrated that the deprotonation of **5a** takes about 300 ns in DNA photolyase. As the pH 7.0 buffer is an efficient proton trap we carried out the laser experiments with **1d** in the absence of buffer (in $CH_3CN/H_2O = 3:1$). These conditions mimic an enzymatic situation slightly better. Under these conditions a 1:1 ratio of **5a**/**5b** was obtained.[10] The enzymatic environment seems to be a less efficient proton trap than the homogeneous acetonitrile/water medium.

The concentration of the two oxidized tryptophan intermediates 40 ns after the laser flash was about 30 %.[12] Thus, tryptophan acts as an efficient electron donor but further ET from tyrosine to the tryptophanyl radical is slow. Harriman and co-workers[13] have determined that the rate of bimolecular ET from a tyrosine residue to a tryptophanyl radical at pH 7.5 (H_2O, 20 °C) is as low as $5 \times 10^5\ M^{-1}s^{-1}$. They explained this result with transition state **6**, in which both reactants are in proximity and the ET is coupled with a proton transfer (Scheme 2). In peptide **1d**, the tyrosine and tryptophan



Scheme 2. PCET between an indolyl radical and phenol.

groups are separated from each other by the triproline spacer so that a transition state such as **6** cannot be reached during an intramolecular reaction. Brettel and co-workers[5] have already suggested that tryptophan can act as an efficient relay amino acid only if the proton of the tryptophan radical cation is hydrogen bonded within the peptide, so that it can easily be transferred back if needed for the next proton-coupled ET (PCET) step.[14] This situation is similar to ET over a long distance in double-stranded DNA, where the proton of the purine radical cation remains within the DNA because of its hydrogen bond to the adjacent Watson–Crick base.[15]

Cysteine, whose oxidation potential of 0.92 V versus NHE would be suitable for ET processes,[16] should also be an inefficient relay amino acid because it will be deprotonated, similar to tryptophan, during oxidation. However, experiments with **1e** demonstrated that cysteine acts as a relay amino acid, with 15 % of the tyrosyl radical **3e** being detected 40 ns after the laser flash (Table 1, entry 5). This result is surprising, and we suggest that the proton transfer during the ET process might be mediated by the surrounding water (Figure 1). If this is the case, D_2O should slow down the reaction because of the H/D isotope effect. Therefore, experiments with **1e** in the presence of D_2O ($CH_3CN/D_2O = 3:1$) were carried out. Under these conditions, the yield of the tyrosyl radical decreased from 15 % (Table 1, entry 5) to about 7–8 %. This isotope effect of about 2 is in accord with the suggestion of a water-mediated PCET (Figure 1). Such a water-mediated reaction should be less favorable with the more hydrophobic tryptophan. In addition, the electron distribution at the thiyl radical allows more pathways for the proton transfer than does an indolyl radical (see, for example, **6**).

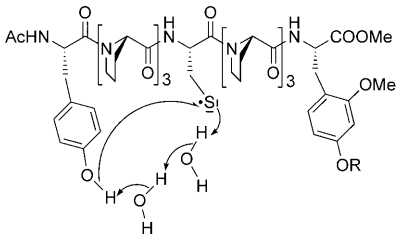
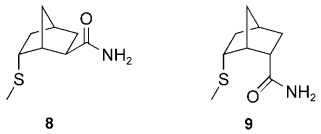


Figure 1. Water-mediated PCET between a cysteinyl radical and tyrosine.

The other natural S-containing amino acid, methionine, also gave surprising results. The high redox potential of a thioether such as dimethylsulfide (1.66 V versus NHE)[16] should make the first ET step in **2f** endergonic, because the redox potential of the electron acceptor (C-terminal amino acid) is about 1.3 V versus NHE.[9] Nevertheless, methionine[17] turned out to be an efficient relay amino acid, with 20 % of tyrosyl radical **3f** being generated 40 ns after the laser flash (Table 1, entry 6). This finding can be explained by a neighboring group effect of the adjacent amide function, which has been demonstrated, for example, by the norbornene systems **8** and **9**: the *endo* amide group in **9** reduces the redox potential by 0.55 V compared to that of **8**.[18] Schöneich



and co-workers[16] have studied such an effect in detail and suggested that the stabilization by a neighboring amide group makes methionine a target for oxidative stress.

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