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Electron Relay Race in Peptides

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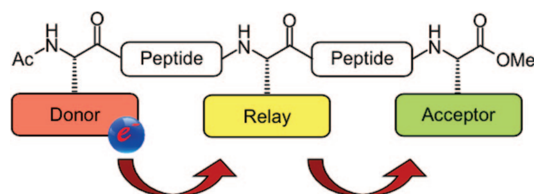

A peptide assay was developed that allows the measurement of electron-transfer (ET) efficiencies in peptides. It turns out that two-step ET processes are faster than single-step reactions. This requires relay amino acids with appropriate redox potentials. Not only aromatic but also sulfur-containing aliphatic amino acids can act as stepping stones for the charge. With tryptophan, histidine, and cysteine the reaction is a more complex proton-coupled ET.

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Introduction

Long-range electron transfer (ET) through proteins and enzymes is a fundamental reaction for all living organisms. Thus, in energy conversion processes like photosynthesis and respiration, multiprotein assemblies control ET through the cell membrane over more than 40 Å.[1] This stimulates proton transfer that drives the synthesis of ATP. An example for long-distance ET through enzymes that catalyze metabolic conversions is the biosynthesis of deoxyribonucleotide from ribonucleotide where ET over more than 30 Å occurs through the enzyme ribonucleotide reductase.[2] The question arises how electrons (or electron holes) can travel through proteins over these long distances with biological relevant rates. According to the Marcus equation, ET rates decrease in a logarithmic way with increasing distance Δr of the ET step (eq 1).[3,4]

$$k = ae^{-\beta\Delta r} \qquad (1)$$

Gray and Winkler have measured the attenuation parameter β in peptides,[5] and Beratan and Onunchic et al. developed a theoretical model for these one-step (superexchange) ET reactions.[6] According to their experimental and theoretical work, the attenuation factor β in peptides is at least 1.0 $Å^{-1}$. As a consequence, rates for one-step ET through peptides decrease dramatically with the distance, and ET over more than 20 or 25 Å should be too slow for biological systems.[7] In contrast to this expectation, ET in biologically important peptides can occur over even longer distances. For an understanding of this long-distance ET in proteins it is helpful to recall the situation in DNA. We have shown that ET through double-stranded DNA oligomers in aqueous solution takes place by a hopping mechanism.[8] According to this mechanism, ET over long distances does not proceed in a single step but in a multistep process. Instead of one slow ET step over a long distance, the reaction occurs in several faster steps over shorter distances; each of them is a superexchange reaction. A condition for such a mechanism is that certain subunits or functional groups can play the role of relay stations where the charge resides for a short time before the ET continues by the next hopping step. In DNA, the heterocyclic bases are the relay stations.[8] From these ET studies in double-stranded DNA one can conclude that long-distance ET through peptides could also occur via a hopping reaction, if the peptides carry relay stations with appropriate redox potentials. This is the case, for example, in photosynthesis and respiration where the charge can hop between cofactors, which are bound to the membrane proteins.[1] But in the enzyme ribonucleotide reductase cofactors that assist charge hopping from the donor to the acceptor are missing, and

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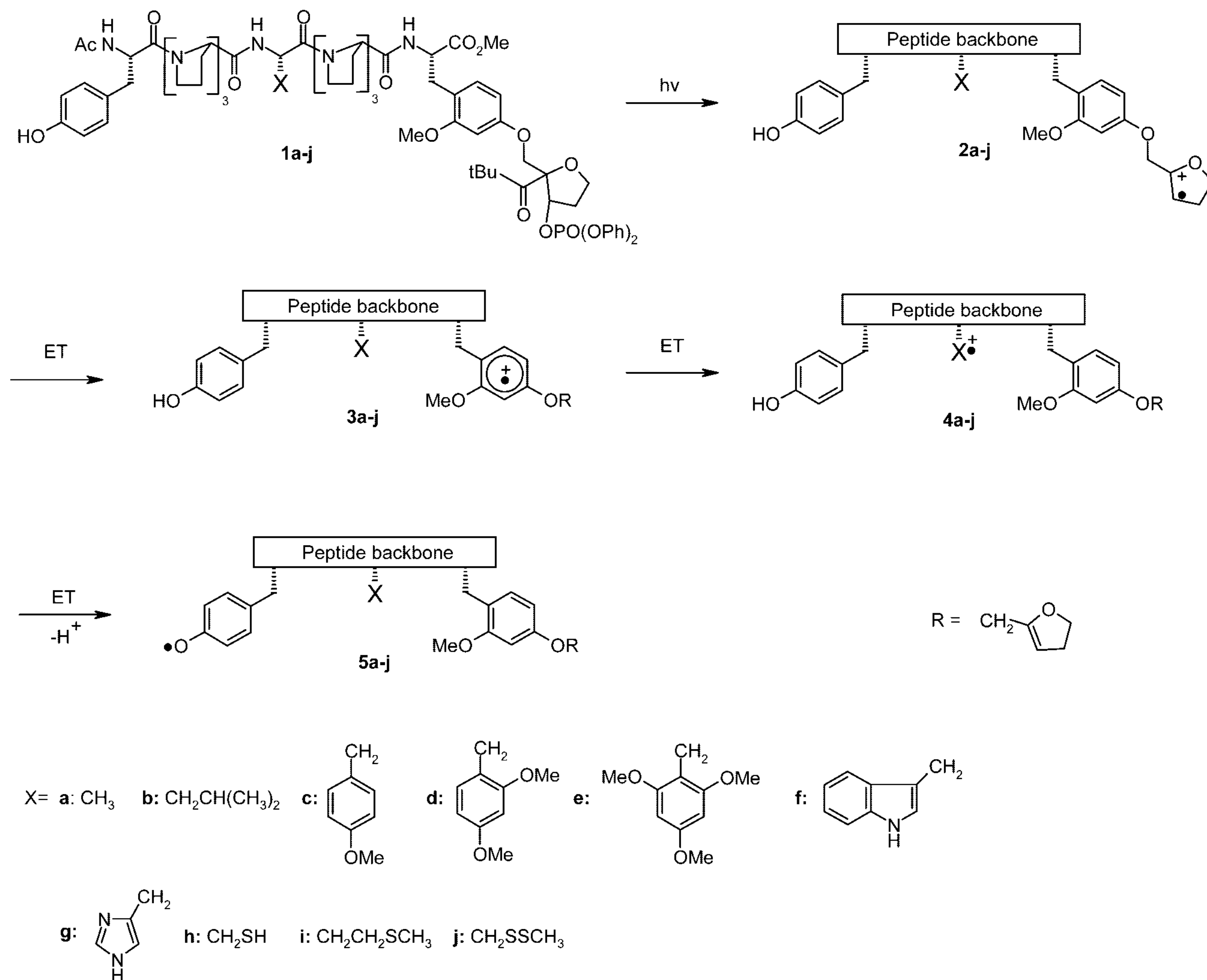
SCHEME 1



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one has to search for amino acids that can act directly as relay stations.[2] Appropriate candidates are, for example, tyrosine and tryptophan, which carry side chains of low oxidation potentials, and these two amino acids are taken advantage of for the explanation of long-distance charge transfer through proteins by a hopping mechanism. This has been brilliantly illustrated by Brettel et al. (photolyase),[9] Stubbe et al. (ribonucleotide reductase),[2,10] as well as Gray and Winkler et al. (azurin).[11]

Peptide Assay as a Stopwatch

In order to find out which amino acids serve as a relays for long-distance ET we have synthesized an assay that can be used as a stopwatch.[12] The basic idea was to develop a peptide, which contains a charge injection system at the C-terminal amino acid, a relay amino acid with a side chain X, and an N-terminal amino acid as electron donor (D). By laser irradiation a radical cation shall be injected into the side chain of the C-terminal amino acid that then acts as electron acceptor A. This triggers ET from the electron donor D, and the influence of the side chain X on the rate of the electron transfer shall be measured following the UV absorptions of the radicals or radical cations of D, A, and X. An assay that can be used as a stopwatch for long-distance ET must follow several conditions: (1) Generation of the acceptor radical cation has to be faster than ET through the peptide. (2) The intermediate radicals and radical cations, respectively, should differ in their UV spectra. (3) Intramolecular ET through the peptide has to be faster than intermolecular ET between two peptides. (4) The peptides separating A and D from the side chain X should be rigid in order to avoid direct contact between the functional amino accids.

We developed model peptide **1** that fulfills all four conditions (Scheme 1).[12,13] The charge is injected by laser irradiation of the ketone group that generates a tetrahydrofuranyl radical. Subsequent elimination of phosphate leads to an enolether radical cation **2** that generates peptide **3** carrying a radical cation as electron acceptor A. If the side chain X can act as a relay station, it will be oxidized by ET producing a new reactive intermediate **4**. Phenol at the N-terminal amino acid tyrosine is the final electron donor (D) and yields phenoxyl radical **5** by deprotonation. The overall ET is exothermic by 0.1 eV because the redox potentials of dialkoxyphenylalanine and tyrosine are 1.03 and 0.93 eV, respectively.[13] Intermediates **3** (start of ET through the peptide) and **5** (end of ET) can be detected simultaneously as their UV maxima are at 450 and 410 nm. Their extinction coefficients are 4000 and 3000 M^{-1} cm^{-1}, respectively; therefore the experiments have to be carried out in 3−8 mM solutions. As a consequence, intermolecular ET might compete in a significant way. Our test experiments had

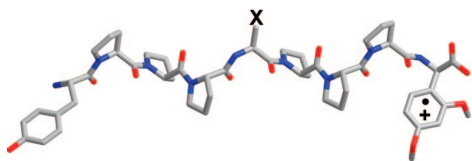

FIGURE 1. PPII conformation of the peptide assay **3**.

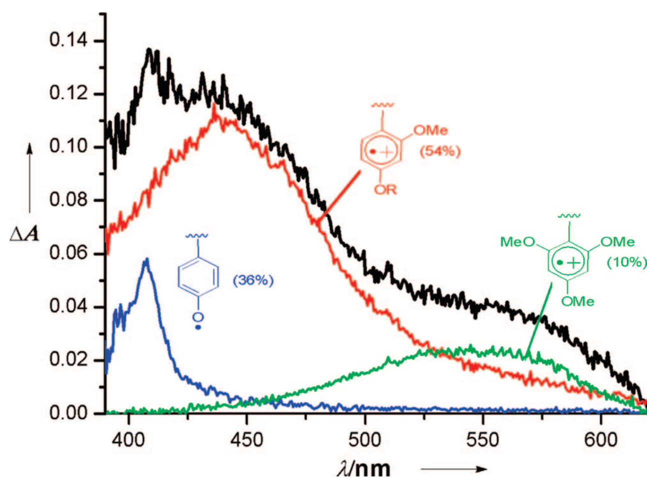

FIGURE 2. UV spectrum (black) 40 ns after the laser flash of **1e**, which is fitted by the red, blue, and green lines (reactive intermediates).

shown that about 6% of intermolecular ET already occurred 40 ns after the laser flash. Because of the rate coefficient of 3×10^{-8} s^{-1} for the charge injection step (formation of **3**), it is not useful to start the ET measurements much earlier than 40 ns after the laser flash. Therefore, we decided to carry out the measurements after 40 ns and subtracted 6% from the tyrosyl radical concentration in order to determine intramolecular ET through the peptide.[13] The triprolines of peptide **1** form rigid PPII helices as demonstrated by their CD spectra.[12] In this PPII conformation, the distances between A and D is about 20 Å. A picture of assay **3**, which implements the results of structural studies of Wennemers et al. on similar polyproline peptides, is shown in Figure 1.[14]

In order learn which side chain X renders an amino acid to a relay amino acid, we determined the influence of X on the yield of the phenoxyl radical **5**. Peptides **1a**−**j** were irradiated, and the UV spectra of the reactive intermediates were measured 40 ns after the laser flash. Experiments with alanine and homoleucine but also with the aromatic amino acid 4-methoxyphenylalanine yielded not more than 1% of phenoxy radicals **5a**−**c** by intramolecular ET (Table 1). This changed dramatically when aromatic side chains X of lower redox potentials were introduced.

Thus, with 2,4-dimethoxy- or 2,4,6-trimethoxyphenylalanine the yield of tyrosyl radicals **5d**,**e**, generated by intramolecular ET 40 ns after the laser flash, increased to 20−30% (Table 1). A direct proof for the action of 2,4,6-trimethoxyalanine as a relay amino acid was the detection of 10% of its radical cation **4e** (Figure 2).[13] Thus, ET through peptide **3e** is a two-step hopping reaction, which is about 30 times faster than the one-step superexchange ET as in **3a**−**c**. These experiments show that peptide system **1** is an appropriate assay to distinguish between superexchange and hopping ET, and we have used this assay to determine, which side chains X allow amino acids to become relay stations in these processes.

TABLE 1. Yield of Tyrosyl Radical Generated 40 ns after the Laser Flash of Peptide 1a−j by Intramolecular ET

peptide **1**	relay amino acid	tyrosyl radical **5** (%)	oxidated relay amino acid (%)
a	alanine	≤1	
b	homoleucine	≤1	
c	4-methoxyphenylalanine	≤1	
d	2,4-dimethoxyphenylalanine	20	
e	2,4,6-trimethoxyphenylalanine	30	10
f	tryptophan	1−2	30
g	histidine	5	
h	cysteine	15	
i	methionine	20	
j	cystin model	20	

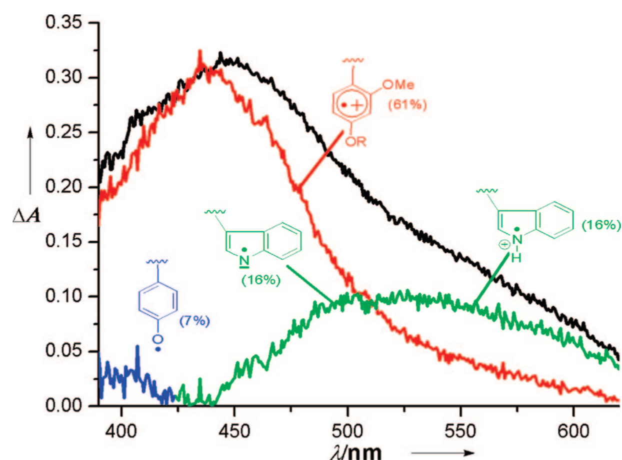

FIGURE 3. UV spectrum (black) 40 ns after the laser flash of **1f**. Subtraction by the red line (electron acceptor) leads to blue and green lines (oxidized electron donor and relay amino acid, respectively).

Proton-Coupled ET with Tryptophan, Histidine, and Cysteine as Relay Amino Acids

ET using tryptophan as relay amino acid has been suggested by Brettel et al. to explain the regeneration of the DNA repair enzyme photolyase[9] by Stubbe et al. to make long-distance ET between a phenoxyl radical and cysteine in ribonucleotide reductase plausible[2] and by Gray et al. to understand fast ET in an azurin model system.[11] In order to see how tryptophan acts as a relay amino acid, we carried out experiments with peptide system **3f** (Figure 3). The results were surprising.[15] After 40 ns of the laser flash about 30% of the oxidized tryptophan was formed as a 1:1 mixture of the tryptophyl radical **6a** (λ_{max} = 520 nm) and its protonated form **6b** (λ_{max} = 570 nm) in a buffer-free solution. The ratio between the base **6a** and the acid **6b** could be influenced by working in different buffer solutions. Thus, at pH = 3.8 the **6a/6b** ratio was 2:1 and reached 10:1 at pH = 7.0.[15]

But only 1−2% of tyrosyl radicals **5f** were generated by intramolecular ET, which is not much higher than with alanine or homoleucine as central amino acids (Table 1). This is surprising if one compares the result with experiments of trimethoxyphenylalanine as relay amino acid (**1e**), which has about the same redox potential as tryptophan and which leads to 30% of the tyrosyl radical and only 10% of the oxidized relay amino acid (Figures 2 and 3). In both cases, a consecutive reaction occurs. With trimethoxyphenylalanine as relay amino acid (**3e**), the second ET step (**4e** to **5e**) is 5 times faster than the first ET step (**3e** to **4e**).[13] This contrasts the tryptophan experiments where the first step (**3f** to **4f**) is at least 10 times faster than the second step (**4f** to **5f**). Why is the oxidation of

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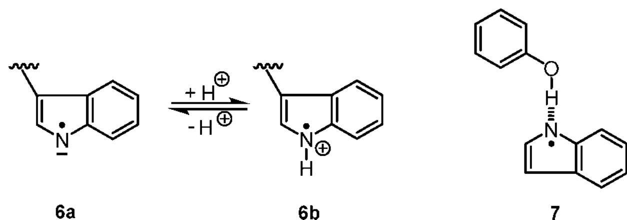
phenol by the tryptophyl radical so slow? Bimolecular reactions with tryptophan and tyrosine in solution gives insight into this process. Simic et al. have observed that the intermolecular ET rate constant between a tryptophyl radical and tyrosine is $4 \times 10^5\ s^{-1}\ M^{-1}$, which makes this reaction much slower than diffusion.[16] The reason is that electron transfer can occur only if at the same time a proton is transferred from the phenol side chain to the indolyl radical, which requires a constrained transition state **7**.

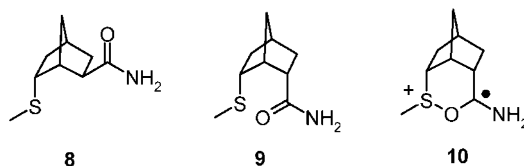


A transition state like **7** is impossible during intramolecular ET in our peptide assay where tyrosine and tryptophan are separated from each other by a triproline spacer. Under which conditions can tryptophan act as a relay amino acid in long distance ET? Looking at the hopping mechanism in DNA could give an answer. During ET through double-stranded DNA the guanine base acts as relay station, although the pK_a of the guanine radical cation is 4.7 and should be deprotonated in water. But in double-stranded DNA the cytosine of the complementary strand traps the proton and delivers it back when the hopping continues. Mismatch studies and a deuterium isotope effect of 3 confirmed such a proton-coupled ET in double-stranded DNA.[17] In a similar way, tryptophan can act as an efficient relay amino acid if the proton of the tryptophan radical cation forms a H-bound to an adjacent functional group of the peptide. Actually, Brettel et al. have explained the reductive regeneration of the DNA photolyase by a mechanism with tryptophan as electron donor.[9] The complementary positive charge is then transferred from the reaction center using other tryptophanes that act as relay amino acids as long as they are protected against water. The last tryptophan in this series is exposed to water and loses its proton. This step makes the charge separation irreversible.

Such a proton-coupled ET should also make histidine and cysteine to uncertain relay amino acids because they can also loose a proton after oxidation. But surprisingly with peptides **1g** (histidine) and **1h** (cysteine) the tyrosyl radicals **5g** and **5h** are formed already in 5 and 15%, respectively, by intramolecular ET 40 ns after the laser flash (Table 1). It is highly likely that the surrounding water enables the proton transfer from tyrosine to the histidyl and especially the cysteinyl radicals. Such a water-mediated process should be faster with amino acids, carrying hydrophilic side chains like mercaptan than with the more hydrophobic indole. In addition, proton transfer to a mercaptyl radical can occur via a larger number of trajectories than proton transfer to the indolyl radical (see transition state **7**). Thus, tryptophan, histidine, and cysteine undergo more complex proton-coupled ET reactions. The success of these amino acids in an electron relay race depends upon the possibility of reversible proton transfer in the peptides.

Sulfur-Containing Aliphatic Relay Amino Acids

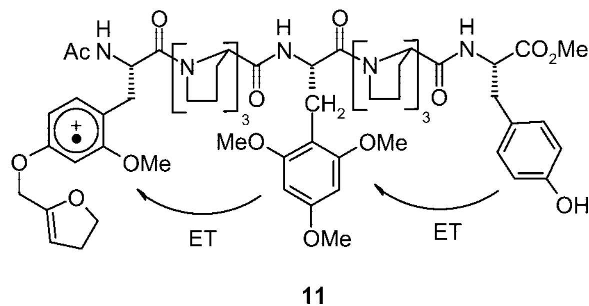
The mercaptan redox potential of 0.93 V makes cysteine to an aliphatic relay amino acid in peptides (**1h** in Table 1).[15] But our experiments have demonstrated that also methionine is an efficient stepping stone for a multistep ET (**1i** in Table 1),[15] although the redox potential of an thioether[18] is too high to become oxidized by the electron acceptor in peptide **3**. Nevertheless, 20% of the tyrosyl radical are formed by intramolecular ET 40 ns after the laser flash of **1i**. This can be explained by a neighbor group effect. In isomer **8** the amide is attached exo to norbornane and is far away from the endo thioether group. But in the endoisomer **9** where the amide and thioether groups are close to each other the redox potential is reduced to 0.85 V, because the capto-dative stabilized radical cation **10** can be generated during oxidation. Such a neighbor group effect is also possible with methionine as relay amino acid in peptide **1i**. Actually, Schöneich et al. have discussed such a neighbor group participation in peptides and predicted that this effect makes methionine to a target of oxidative stress.[19]



We have also studied ET in peptide **1j**, a model for cystine as relay amino acid. As expected from the redox potential of disulfides[20] also cystine turned out to be an efficient relay amino acid (Table 1). These examples demonstrate that not only aromatic amino acids like tyrosine, tryptophan, or histidine but also the sulfur containing aliphatic amino acids cysteine, cystine, and methionine can act as relay amino acids in the electron relay through peptides. It is highly likely that nature has used this effect for long distance ET processes, although to our knowledge this has not been detected yet.

Determination of Molecular Dipoles in Peptides by ET

Because of their amide (carbonyl) groups, peptides can have large dipole moments that influence the ET rates. Thus, the orientation of the carbonyl groups in an α-helix produces an dipole with its positive end at the N-terminal and its negative end at the C-terminal amino acid. In accord with this direction of the molecular dipole, Fox et al. have measured a much faster ET from the C- to the N-terminal site of an α-helix compared to the opposite direction.[21] Peptide assay **1** has the conformation of a PPII helix where the carbonyl groups are nearly orthogonal to the peptide backbone (Figure 1). For such a conformation opposing dipole moments have been calculated.[22]



We therefore generated radical cation **11** from its respective precursor and compared its ET rate with that of **3e**. In **11**, electrons migrate from the C-terminal to the N-terminal amino acid, which is opposite to that in **3e**. Experiments showed that

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ET in **3e** is about 5 times faster than in **11**. Thus, in PPII helices the C-terminal amino acid is the positive and the N-terminal amino acid is the negative end of the molecular dipole so that its direction in α-helices is opposite to that in PPII helices.

Conclusion

Long-distance ET through peptides occurs in a multistep hopping process if relay amino acids with appropriate redox potentials are present. Such a relay race of electrons in peptides is much faster than single-step superexchange. Not only aromatic but also sulfur-containing aliphatic amino acids can act as relay stations. If the oxidized relay amino acid loses a proton the reactions are more complex because the conditions have to facilitate not only an electron but also a proton transfer. Furthermore, ET rates depend upon the molecular dipole moments of the peptides; thus ET can be used as analytic tool to determine peptide conformations.

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