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The Search for Relay Stations. Long-distance Electron Transfer in Peptides

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Abstract: Nature uses peptide aggregates as soft materials for electron transfer over long distances. These reactions occur in a multistep hopping reaction with various functional groups as relay stations that are located in the side chain and in the backbone of the peptides.

Keywords: Electron transfer · Hopping · Mineral respiration · Peptide

1. Introduction

Peptides play various roles in living organisms. One of their tasks is the transport of electrons over long distances.[1] They connect electron donors and electron acceptors with each other, and mediate redox reactions between them. In past years it became obvious that bacterial pili, aggregates that are built up by non-covalent interactions of large peptides, are excellent materials for electron transfer (ET) over long distances (μm and more).[2] During 'mineral respiration' these pili connect the inner cell membrane with minerals or metal ions that are situated outside of the cell. In this way, extracellular metal ions drive metabolic processes within the cell. In some cases these pili do not contain cofactors, which could act as intermediate redox stations, so that only the amino acids of the peptides transport the electrons over μm distances. According to the Marcus theory the influence of the distance Δr on ET rates k_{ET} is described by Eqn. (1), where k_0 is the rate at contact distance between donor and acceptor and β is a material parameter.[3]

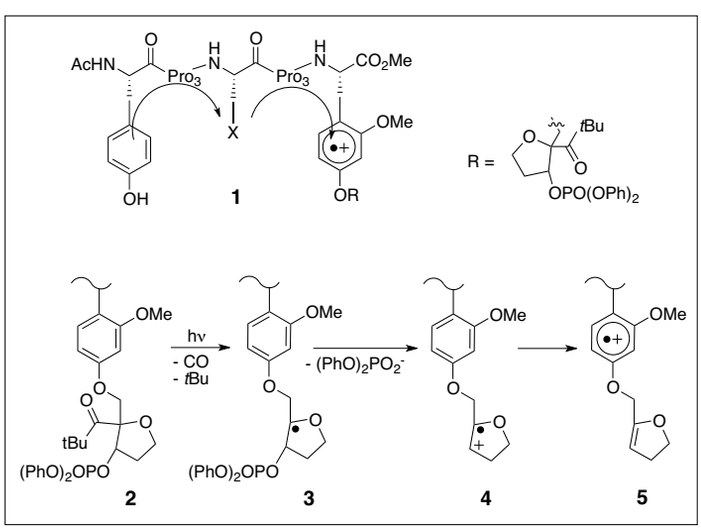
$$k_{ET} = k_0 \cdot exp(-\beta\Delta r) \quad (1)$$

It was shown that single step ET through peptides over more than 2 nm is too slow for biological systems.[4] Therefore ET over μm distances has to follow a different mechanism. We have developed an assay that allows studies on ET in peptides, and it became obvious that long-distance ET through peptides are multistep hopping reactions.[5] This is reminiscent of ET through DNA, where two base pairs of the nucleic acids act as stepping stones for a multistep hopping reaction.[6] Instead of one long and therefore very slow ET, the reaction occurs in several short and therefore very fast ET steps. The overall reaction can be described as diffusion of the charge between stepping stones. Compared to DNA, which has only two different base pairs as stepping stones, peptides offer more relay stations for multistep ET processes. This variety of relay stations in peptides and proteins is very important as, in most cases, the peptide backbone and the ET pathway point in different directions. In these situations ET follows the peptide backbone only over short distances (secondary structure plays a role), and hopping occurs between relay stations situated at very different sites of the peptide (primary structure). Thus electrons take a shortcut, they hop through the tertiary structure using several relay stations, which are located between the electron donor and the electron acceptor. In addition, peptides form aggregates (quaternary structure) so that ET hopping also occurs between relay stations of different peptides. This situation contrasts dramatically with ET in DNA where the relay stations (stepping stones) are lined up like pearls on a string, and aggregation between DNA double strands does not occur (charges!). Thus, the DNA backbone and ET point in the same direction.

2. Amino Acids Side Chains

2.1 *Relay Amino Acids*

In order to answer the question, which amino acid acts as a relay station for ET in peptides, we have developed an assay which contains the radical cation **1** of dialkoxylphenylalanine as electron acceptor, tyrosine as the electron donor, and prolines as spacers that mediate ET from the donor to the acceptor.[5] The electron acceptor is generated by a laser flash of a precursor, which yields the aromatic radical cation (**2**→**5**, Scheme 1).[7]



Scheme 1. Assay for the measurement of ET through peptides.

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Radical cation **1** has an oxidation potential of 1.3 V *vs.* NHE so that the electron acceptor cannot oxidize the proline spacers, which therefore act as a medium and not as stepping stone of the ET process. In between the donor and the acceptor of **1** we incorporated an amino acid with the side chain X. If this amino acid is a relay station of the ET reaction, the rate will be speeded up, because a slow one-step ET (superexchange reaction) over long distances is switched to two fast ET steps (hopping reaction) over shorter distances. In the first reaction step side chain X acts as electron donor and is oxidized to its radical cation. In the subsequent step this radical cation acts as electron acceptor so that ET continues. In some cases the radical cation of the side chain X was observed. We have carried out laser experiments with this assay and discovered that not only the aromatic amino acids tyrosine, tryptophan and histidine[5a] but also the sulfur-containing amino acids cysteine, cystine and methionine are stepping stones for a hopping reaction (Fig. 1).[5c,8] These amino acids have been named by us as relay amino acids.

Amino acids with N–H, O–H or S–H bonds become acids during oxidation to their radical cations. If they lose a proton their oxidation potentials decrease, which makes the oxidation of these amino acids easier (proton coupled ET) but it slows down or even stops the next ET step. We have observed a similar situation in electron hole processes through DNA, but in double-stranded DNA the proton remains H-bridged in the base-paired biopolymer.[9] Trapping of the proton within peptides is also possible if appropriate H-acceptors are situated nearby.

2.2 *Neighbor Group Effects*

Surprisingly also methionine is a relay amino acid, although the oxidation potential of a simple dialkylthioether is higher than 1.3 V. It turned out that the neighboring amide group stabilizes the radical cation (Fig. 1, **6**→**7**).[8,10] A phenyl group can also stabilize a methionine radical cation by a neighbor group effect.[11] Our experiments with alkoxyphenylalanines indicate that in peptides also an aryl radical cation can be stabilized.[12] As test amino acids we used dimethoxy- and trimethoxyphenylalanine derivatives **11** and **8**, respectively: a) their oxidation potentials differ by 10 mV in favor of the trimethoxyphenylalanine, and b) their radical cations can be easily detected by UV-vis spectroscopy.[5a,b] Starting with a 1:1 mixture of the amino acid derivatives **8** and **11**, and generating small amounts of the radical cation **9** by a laser flash,[13] led to an equilibrium of the aromatic radical cations **9**:**10** = 40:60 (Scheme 2).[14] This is in accord with the small difference of the redox potentials of the amino acids favoring the trimethoxyphenylalanine radical cation.

If the two methoxyphenylalanines are part of the same peptide, separated by one proline, the equilibrium between the radical cations is **12**:**13** = 65:35, that is in favor of the dimethoxyphenyl side chain (Scheme 2). Obviously, the aromatic radical cations in this peptide interact with the peptide. We have quantified this interaction in equilibration experiments with trimethoyxyphenylalanine (**8**). From equimolar amounts of peptide **15** and amino acid **8** we generated in a laser flash small amounts of the radical cations and measured an equilibrium of **14**:**10** = 80:20 (Scheme 3).[14] Only 20% of the charge is transferred from the peptide radical cation **14** to trimethoxyphenylalanine (**8**). The equilibrium constant is 0.25 (Scheme 3) and therefore 6 times smaller than the equilibrium constant of 1.5 (Scheme 2) using the amino acid radical cation **9** instead of the peptide **14**.

The rates of irreversible trapping experiments with tyrosine also point to a stabilizing effect of the positive charge in peptide **14**.[15] The radical cation of peptide **14** reacts two times slower with tyrosine ($k = 2.3 \times 10^8$ s^{-1}) than the radical cation of amino acid **9** ($k = 4.6 \times 10^8$ s^{-1}). This is analogous to thermodynamic and kinetic effects of radical cations in DNA, where a guanine radical cation is stabilized by an adjacent guanine by a factor of 8, and their formation rate is increased by a factor of two.[16]

3. Peptide Backbone

ET processes through peptides and proteins are influenced dramatically by their secondary structure. A pronounced effect is exerted by α- and 3_{10}-helices where the carbonyl groups point from the N-terminal to the C-terminal end of the peptide, thus they display dipole moments that increase with the lengths of the helices. A consequence of these large dipole moments is that the reduction potentials of the amide groups at the C-terminal ends might decrease to such an extent that these amide groups could become relay stations in ET processes.[17] We have measured this effect in peptide **16**, where a fast decrease of the aromatic radical cation (electron acceptor) can be explained with an oxidation of the C-terminal amide group (Scheme 4).[18]

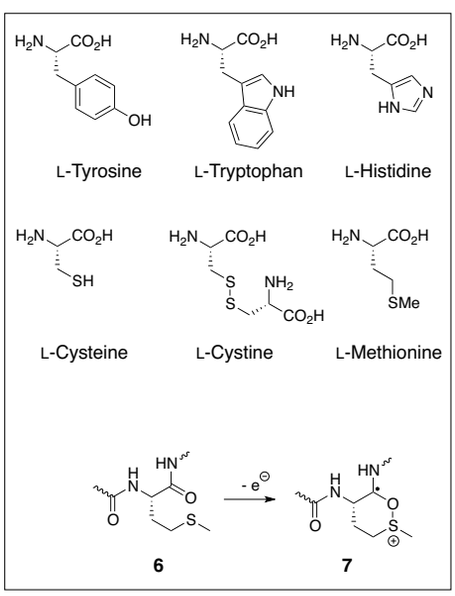
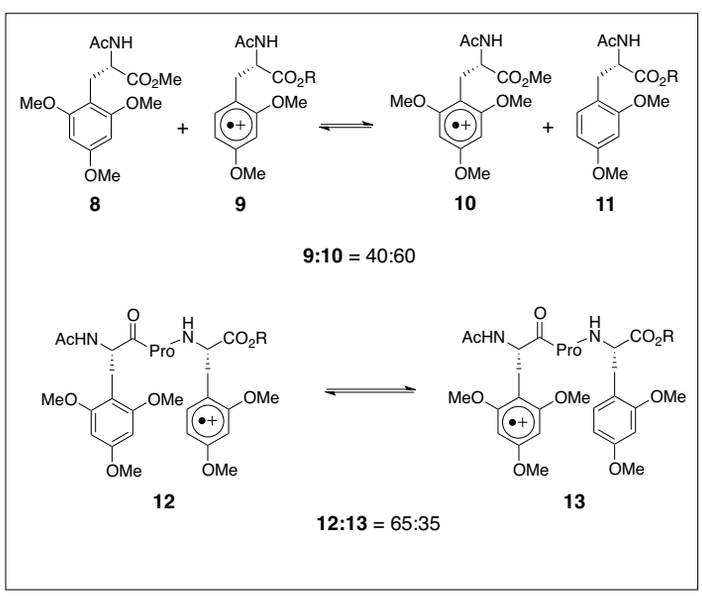
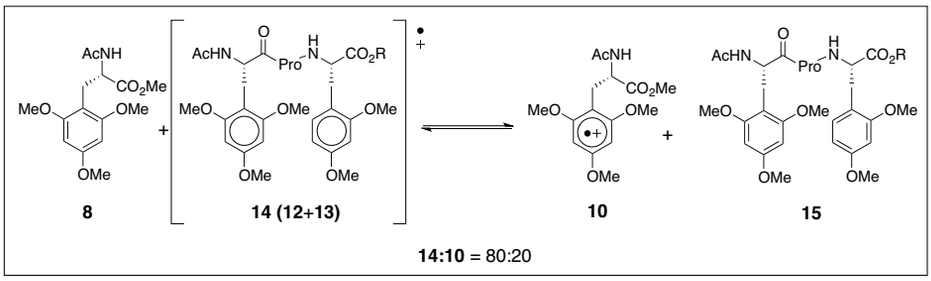


Fig. 1. Amino acids that function as relay amino acids.



Scheme 2. Equilibrium of aromatic radical cations.



Scheme 3. Equilibrium of aromatic radical cations.

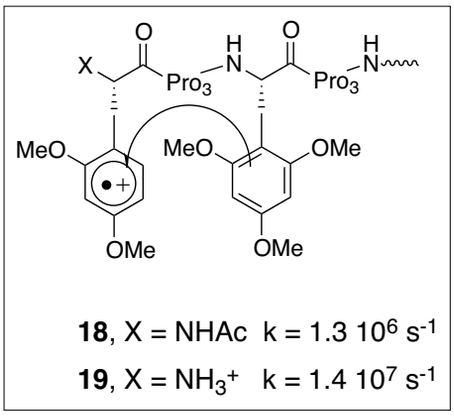


Fig. 2. Influence of a positive charge on ET rates.

Subsequent hopping of the charge using other amide groups (relay stations) of the peptide leads to a fast generation of the tyrosyl radical (oxidation of the electron donor). We conclude that in peptides of these secondary structures the backbone is not only a spacer between donor and acceptor as their amide groups might become stepping stones of a hopping process. These stepping stones speed up ET processes within and between peptides.

4. Charges

A further feature of peptides and proteins is that they can control ET processes using charged amino acids. We have measured this effect introducing a positive charge into peptide **18** by deprotection of its N-terminal end (**18**→**19**).[19] In peptide **19** two positive charges are in short distance to each other, which leads to Coulomb repulsion and increases the oxidation potential. As a consequence the ET reaction, which neutralizes the charge at the N-terminal side chain, is increased by one order of magnitude (Fig. 2). In a similar way the dipole moments of peptides influence ET rates even if they do not change the mechanism from a one-step (superexchange) to a multistep (hopping) mechanism.[19]

5. Prospects

Our laser experiments have demonstrated that peptides can control ET rates by their side chains (primary structure) and conformations (secondary structure), as well as neighbor group effects and charges. These interactions make a direct electron migration through the tertiary structure of a large protein possible. The electron hops from the electron donor to the electron acceptor using different peptide sequences of the large protein.

Nature utilizes peptides for long-distance ET processes not only as single molecules but also as soft materials in which the peptides have been self-assembled and formed 'polymers' (quaternary structure). The influence of peptide structures on ET rates that we have described above holds not only for intramolecular ET *within* one peptide molecule but also for intermolecular processes *between* the peptide 'monomers'. The dense packing in peptide aggregates can bring hopping stations close to each other and increase the importance of neighbor group effects. Latest publications on the structure of 'monomers' and 'polymers' used in mineral respiration are in accord with these conclusions.[2b,20]

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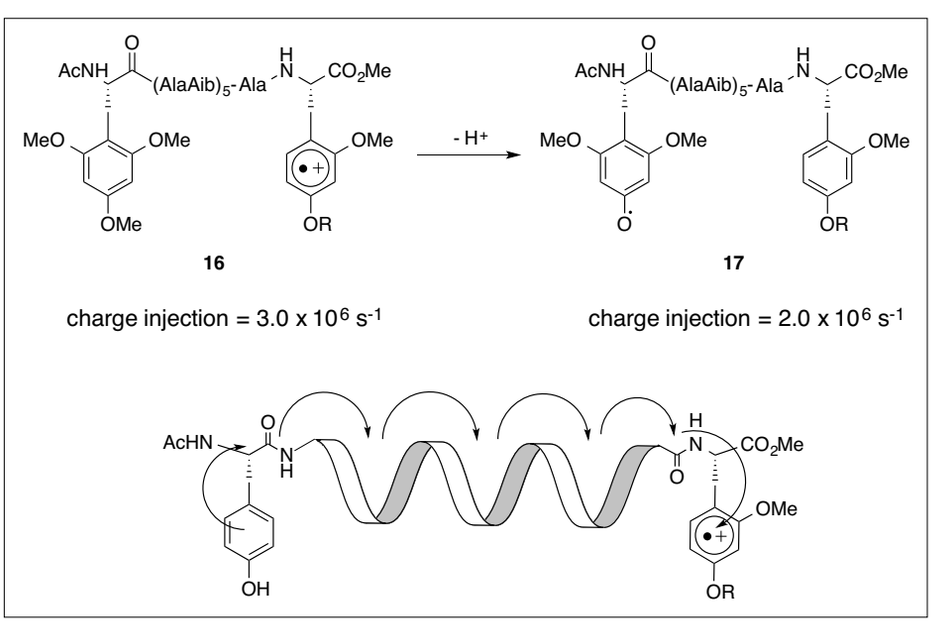

Scheme 4. Electron hopping through α-/3_{10}-helices.

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