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The influence of dipole moments on the mechanism of electron transfer through helical peptides†

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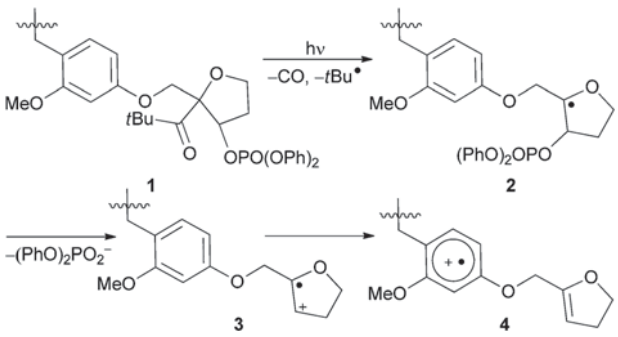
The life time of aromatic radical cations is limited by reactions like β-elimination, dimerization, and addition to the solvent. Here we show that the attachment of such a radical cation to the C-terminal end of an α-/3_{10}-helical peptide further reduces its life time by two orders of magnitude. For PPII-helical peptides, such an effect is only observed if the peptide contains an adjacent electron donor like tyrosine, which enables electron transfer (ET) through the peptide. In order to explain the special role of α-/3_{10}-helical peptides, it is assumed that the aromatic radical cation injects a positive charge into an adjacent amide group. This is in accord with quantum chemical calculations and electrochemical experiments in the literature showing a decrease in the amide redox potentials caused by the dipole moments of long α-/3_{10}-helical peptides. Rate measurements are in accord with a mechanism for a multi-step ET through α-/3_{10}-helical peptides that uses the amide groups or H-bonds as stepping stones.

1. Introduction

Long range electron transfer (ET) through proteins is a fundamental reaction in living organisms playing a role in energy conversion processes like photosynthesis,[1] aerobic,[1] and anaerobic[2] respiration as well as enzymatically driven transformations.[3] In addition, ET through peptides and proteins has become of interest for the development of molecular based electronics.[4] Two mechanisms are discussed to explain long range ET between an electron donor and an electron acceptor. The reaction might either occur *via* bridge-assisted super-exchange (single-step reaction) or by a stepwise, so-called hopping reaction.[5] During superexchange, the amino acids mediate ET and do not undergo a change in their redox states while during hopping, certain amino acids are reversibly oxidized or reduced and serve as relay stations (stepping stones) for the ET process. Important parameters that influence ET mechanisms are peptide conformations,[6,7] peptide flexibility,[8] and redox potentials of the peptide side chains that might become stepping stones for a hopping process if their redox potentials are low enough.[5,9]

Of special interest are α- and 3_{10}-helices as their high dipole moments could strongly change the redox potential of the amide groups of the peptide backbone.[10] According to quantum chemical calculations of Bu and Cukier, α-helical peptides consisting of ten or more amino acids exhibit amide oxidation potentials of the peptide backbone that are as low as those of heterocyclic or sulfur containing side chains.[11] Amino acids containing such side chains are typical relay stations for a multi-step (hopping) ET process.[5,9] One can therefore assume that the low amide oxidation potentials of α-helical peptides might facilitate ET by a hopping mechanism using the amide bonds of the backbone as stepping stones. In fact, Kimura *et al.* recently measured oxidation potentials of about 1.1 V *vs.* NHE for peptides containing eight to sixteen helix-inducing amino acids, alanine (Ala) and aminoisobutyric acid (Aib).[12] From ET experiments with self-assembled monolayers (SAMs), they concluded that "hopping among the amide groups might be involved in long-range ET if oxidation or reduction of the amide groups is energetically available".[12] Recently, we have developed a peptide system carrying the aromatic radical cation **4** with an oxidation potential of 1.3 V *vs.* NHE, which functions as an electron acceptor.[9,13] According to quantum chemical calculations and electrochemical measurements mentioned above, this oxidation potential of **4** should be high enough to oxidize the amide groups of α-helices, which as a consequence might be able to become stepping stones for a hopping reaction.

Herein, we describe kinetic laser experiments, which actually indicate that radical cation **4** injects a positive charge into amide groups located in the backbone of α-/3_{10}-helical peptides.



Scheme 1 Generation of radical cation **4** by laser flash photolysis of its precursor **1**.

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2. Results and discussion

2.1 Generation of the aromatic radical cation

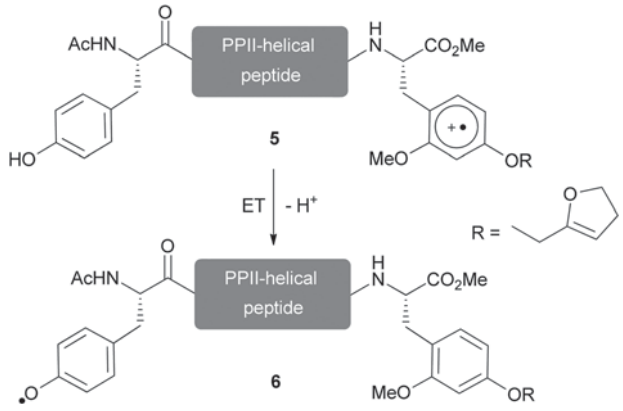
Radical cation **4**, used for the charge injection, was generated by laser flash photolysis of its precursor **1** yielding radical **2** in a Norrish reaction. After heterolytic β-bond cleavage, the enolether radical cation **3** was formed, which oxidized the aromatic ring (**3** → **4**).[9,13]

2.2 Polyproline II-helical peptides

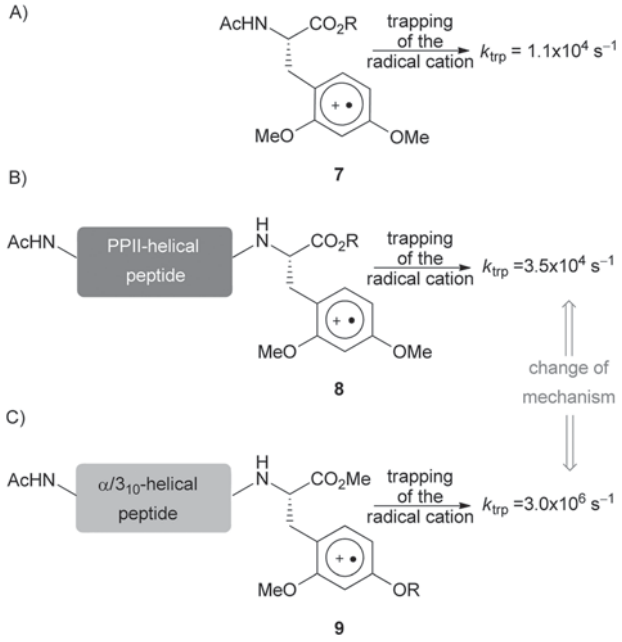
We have recently attached the radical cation precursor **1** to peptides of type **5** and used the generated radical cation as an electron acceptor for the measurement of ET rates. In peptides **5** tyrosine acts as an electron donor, which is in all cases separated from the electron acceptor by several prolines (Scheme 2). Under our reaction conditions, these peptides adopt the secondary structure of a polyproline II (PPII)-helix (see discussion below).[14] During ET (**5** → **6**), the electron acceptor (aromatic radical cation) is reduced and yields the neutral aromatic system, whereas the electron donor (tyrosine) is oxidized and generates the tyrosyl radical. From the disappearance of the radical cation **5** (decrease of its UV/vis absorption, λ_{max} = 450 nm) and the formation of the tyrosyl radical **6** (increase of its UV/vis absorption, λ_{max} = 410 nm) the ET rates through the peptides could be elucidated.[9a,15]

Since aromatic radical cations are reactive intermediates, their decrease is not only caused by ET from electron donors (reduction), they also react by β-bond cleavage,[16] dimerization,[17] and addition to the solvent.[18] We had earlier measured the overall trapping rate (k_{trp}) of the alkoxy substituted aromatic radical cation **7** in the absence of electron donors (Scheme 3).[19] Laser experiments with the respective precursor of **7** (5 mM solutions in CH_3CN/H_2O = 3 : 1) led to an observed pseudo-first order rate of $k_{trp} = 1.1 \times 10^4$ s^{-1} for the decrease of radical cation **7**.[20] Thus, the half-life of the aromatic radical cation in amino acid **7** is $\tau_{1/2} = 0.6 \times 10^{-4}$ s in the absence of electron donors.

In order to learn whether a peptide influences the life time of this radical cation, we incorporated amino acid **7** into peptide **8**, which contains six prolines and two phenylalanines (Scheme 3). Spectroscopic studies and force field calculations have proven



Scheme 2 ET through PPII-helical peptides: the aromatic radical cations **5** act as electron acceptor and are reduced by the electron donor tyrosine, which itself is oxidized and deprotonated to form tyrosyl radicals **6**.



Scheme 3 Pseudo-first order trapping rates (k_{trp}) of (A) radical cation **7**, the radical cation being attached (B) to a PPII-helical peptide (**8**), and (C) to an α-/3_{10}-helical peptide (**9**). The rates were determined from the decreasing UV/vis-signal of the aromatic radical cation (λ_{max} = 450 nm). The amino acid sequence of the PPII-helix is Phe-Pro_3-Phe-Pro_3 and for the α-/3_{10}-helix the sequence is $(Ala\text{-}Aib)_5$-Ala. R is the same as in Scheme 2.

that peptide **8** adopts the conformation of a PPII-helix.[9,21,22] The dipole moments of PPII-helical peptides, which have the positive dipole end at the C-terminal amino acid, are small and they hardly influence the oxidation potential of the amide groups.[22] In simple organic compounds, these amide groups exhibit oxidation potentials in the order of 2 V *vs.* NHE.[23] As the redox potential of the aromatic radical cation **8** is only 1.3 V *vs.* NHE, it should not be able to oxidize the amide groups of this PPII-helix.[9a] Therefore, it is highly unlikely that the life time of the radical cation **8** will be reduced by ET from the adjacent amide groups of the PPII-helical peptide. We generated **8** by laser irradiation of the respective precursor of the radical cation (see Scheme 1) and measured the decrease of its UV/vis signal. The determined trapping rate and half-life of the aromatic radical cation **8** ($k_{trp} = 3.5 \times 10^4$ s^{-1}, $\tau_{1/2} = 0.2 \times 10^{-4}$ s) are slightly higher but in the same order of magnitude as those of amino acid **7** ($k_{trp} = 1.1 \times 10^4$ s^{-1}, $\tau_{1/2} = 0.6 \times 10^{-4}$ s).[20,24] The positive end of the small peptide dipole at the C-terminal amino acid of PPII-helices should slightly increase the reactivity at the nearby aromatic radical cation. This might explain the small rate difference between amino acid **7** and peptide **8**. In contrast to this small rate increase, a radical cation incorporated into PPII-helical peptides **5** that contain nearby electron donors reacts faster by several orders of magnitude.[9a,15] From the rate data described above we concluded that in PPII-helical peptide **8** an amide group cannot be oxidized by a radical cation with an oxidation potential of 1.3 V *vs.* NHE. The decay of the radical cation is caused by trapping of the reactive intermediate by β-bond cleavage,[16] dimerization,[17] and addition to the solvent[18] while an ET process does not occur.

2.3 α-/3_{10}-Helical peptides

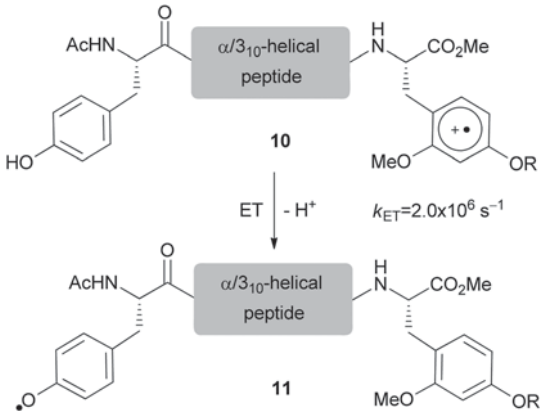
Next, we studied the question whether the lifetime of the aromatic radical cation changes if α-/3_{10}-helical peptides instead of PPII-helical peptides are used. Their conformations differ in several parameters such as torsion angles, H-bonding network, and dipole moments. In PPII-helices, the polar carbonyl groups of the peptide bonds are oriented nearly orthogonal to the peptide backbone, and mainly compensate each other. The resulting small dipoles have their positive dipole at the C-terminus.[22] α-Helical peptides often coexist in one peptide strand with 3_{10}-helices.[25] Both are right-handed helices but they are stabilized by different H-bonding patterns.[26] The α- as well as the 3_{10}-helix are conformations in which the polar carbonyl groups are oriented parallel to the peptide backbone.[27] These peptides exhibit large dipole moments with the negative dipole end at the C-terminus. As a consequence, the oxidation potentials of the amide groups at the C-terminal end of α- and 3_{10}-helical peptides are decreased and for longer peptides the amide groups might be oxidized by an aromatic radical cation with an oxidation potential of 1.3 V *vs.* NHE.[10–12]

We attached the precursor of the aromatic radical cation to peptide **9**, which contains six alanines and five aminoisobutyric acids. The CD-spectrum of this peptide shows that it adopts the conformation of an α-/3_{10}-helix (see ESI†). Radical cation **9** was generated by laser irradiation (1 mM solution of the respective precursor in CH_3CN/H_2O = 3 : 1), and the decrease in its concentration was measured by UV/vis spectroscopy. The absorption of the radical cation (λ_{max} = 450 nm) decreased with a first order rate of $k_{trp} = 3.0 \times 10^6\ s^{-1}$ ($\tau_{1/2} = 0.2 \times 10^{-6}$ s). Thus, the reaction rate of α-/3_{10}-helical peptide **9** is two orders of magnitude higher than that of the respective cation **8** of the PPII-helical peptide ($k_{trp} = 3.5 \times 10^4\ s^{-1}$). The negative dipole end of the α-/3_{10}-helix **9** is in the proximity of the aromatic radical cation. Coulomb stabilization between the opposite charges should decrease the reactivity (deprotonation, reaction with H_2O, dimerization) of the radical cation. Thus, the observed rate increase by two orders of magnitude has to be caused by another reaction. One major difference between peptides **8** and **9** is the decrease in the oxidation potential of the amide groups in the backbone of the α-/3_{10}-helix compared to a PPII-helix (see discussion above). The rate increase by two orders of magnitude of **9** compared to **8** could be caused by ET from the amide group to the radical cation in **9**, which acts as an electron acceptor. As discussed recently by Kimura, an oxidation of the amide group could change the ET mechanism through peptides from a single-step, bridge-assisted superexchange to a stepwise, hopping reaction, in which the amide bonds act as stepping stones.[12] This mechanistic change increases the ET rate because one long, slow reaction step is replaced by several short, fast steps. Therefore, experiments through long α-/3_{10}-helical peptide **10** were carried out.

2.4 Electron transfer through α-/3_{10}-helical peptides

We measured the ET rate (k_{ET}) in peptide **10**, which contains the same helix-inducing sequence as peptide **9** (see ESI†) but with the electron donor tyrosine attached as N-terminal amino acid (Scheme 4).

In peptide **10** the electron donor (tyrosine) and the electron acceptor (aromatic radical cation) are separated from each other by eleven amino acids, so that ET occurs over a long peptide sequence. The radical cation **10** was generated from its respective precursor (see Scheme 1) by a laser flash under the same conditions as described for **9**. From the increase of the UV/vis absorption at 410 nm, the first order formation of the tyrosyl radical **11** was determined to be $k_{ET} = 2.0 \times 10^6\ s^{-1}$. Thus, the formation rate of the tyrosyl radical during the ET process **10** → **11** is slightly slower than the charge injection step of radical cation **9** that does not contain tyrosine as an electron donor.[28] This observation is in agreement with a mechanism in which the aromatic radical cation at the C-terminal end of the α-/3_{10}-helical peptide **10** actually injects a positive charge into the adjacent amide group. This charge injection induces ET through the peptide (**10** → **11**) by a multistep charge hopping using the amide groups of the helical peptide **10** as stepping stones. Recent calculations of Isied *et al.* have shown that in α-/3_{10}-helical peptides the electron coupling between the amide groups is strong.[7] In addition, H-bonds could be used as a pathway for the ET process.[29] These H-bonds function as short cuts and reduce the distance between the electron donor at the N- and the electron acceptor at the C-terminal amino acid. A hopping mechanism is in accord with electrochemical experiments of Kimura who has shown that the distance dependency of the ET rate through very long peptides with amino acid sequences similar to **10** is very weak.[12]



Scheme 4 ET through an α-/3_{10}-helical peptide: the pseudo-first order rate k_{ET} was determined from the increasing UV/vis-signal of the tyrosyl radical **11** (λ_{max} = 410 nm). The amino acid sequence of the α-/3_{10}-helix is (Ala-Aib)$_5$-Ala. R is the same as in Scheme 2.

3. Conclusions

The life time of an aromatic radical cation with an oxidation potential of 1.3 V *vs.* NHE is reduced by two orders of magnitude if it is attached to the C-terminal end of α-/3_{10}-helical peptide **9**. Trapping and ET rate measurements are in accord with a mechanism in which amide groups of the α-/3_{10}-helical backbone are oxidized by the aromatic radical cation. This charge injection is made possible because the large dipole moments of α-/3_{10}-helical peptides reduce the oxidation potentials of the amide groups. The subsequent ET process through the peptide might occur by a hopping mechanism, in which the amide groups and the H-bonds can act as relay stations. Such a hopping reaction, in which the amide groups of the peptide are

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oxidized and act as stepping stones, resembles the function of guanines and adenines in electron–hole hopping processes through DNA.[30,31]

Acknowledgements

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