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Charge Mapping in 3_{10}-Helical Peptide Chains by Oxidation of the Terminal Ferrocenyl Group

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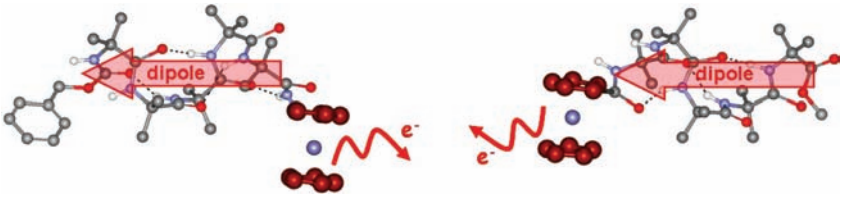
ABSTRACT



Two series of 3_{10}-helical peptides of different lengths and rigidity, based on the strongly foldameric α-aminoisobutyric acid and containing a terminal ferrocenyl unit, have been synthesized. Oxidation-state sensitive spectroscopic tags of helical peptides, the N−H groups, allowed mapping of the charge delocalization triggered by oxidation of the terminal ferrocenyl moiety and were monitored by IR spectroelectrochemistry.

Electron transfer (ET) is one of the fundamental processes in chemistry and biology, and its study using biomolecules has been of great value for our understanding of the energy conversion and mass transduction in Nature and for the development of molecular-based electronics.[1] The spatial organization of the electron donor (D) and electron acceptor (A) groups in proteins and peptides, as well as the dynamics of the ET directing and charge-transmitting properties of the H-bonds between them, critically depends on the 3D-structure of the bridges.[2]

In this context, peptide helices have attracted much attention because their assemblies are universal motifs found in biological ET systems and are considered to play important roles in long-range ET in proteins. The orientation of the carbonyl groups in a peptide helix produces a macrodipole with its positive end at the N-terminal and its negative end at the C-terminal amino acid. Studies have revealed a much faster ET from the C- to the N-terminal site of an α-helix compared to the opposite direction.[3] Investigations have also demonstrated that the ET mechanism switches from a simple electron tunneling for short distances to another mechanism, characterized by a very shallow distance-dependence, for longer distances.[4]

However, the debate about the mechanism of ET through helical peptides is still open.[4,5]

In recent years, increasing interest has been focused on the synthesis and study of ferrocenyl (Fc) peptides. The conjugation of organometallic compounds with biomolecules such as amino acids, peptides, and DNA has provided novel systems that reflect properties of both Fc and the biological moieties. Fc-peptides have been exploited as

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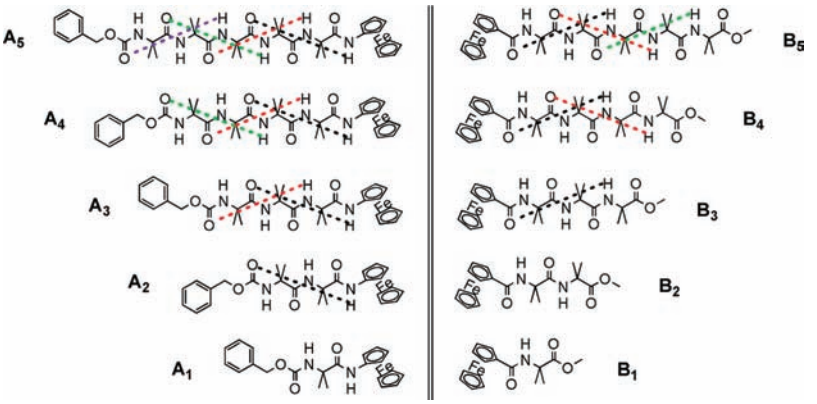

Figure 1. Series $\mathbf{A}_n$ and $\mathbf{B}_n$ of peptides of different length (n = 1−5) bearing a terminal ferrocenyl group.

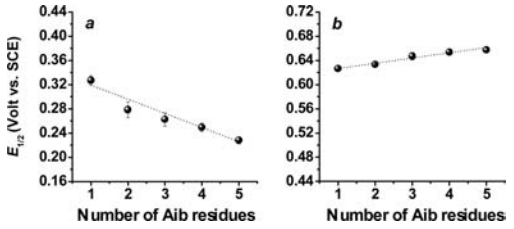

Figure 2. Redox potentials of the Z−$(Aib)_n$−NH−Fc ($\mathbf{A}_n$, n = 1−5) and Fc−CO−$(Aib)_n$−OMe ($\mathbf{B}_n$, n = 1−5) peptides. Potentials were recorded using a 0.5 mm-diameter gold disk electrode in CH_2Cl_2 with 0.1 M nBu$_4$NPF$_6$ as the supporting electrolyte.

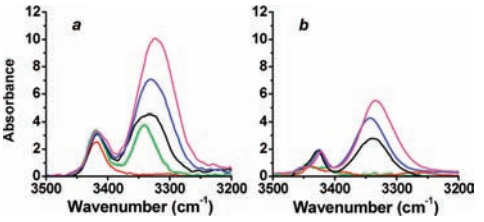

Figure 3. FT-IR absorption spectra in the N−H stretching region of the $\mathbf{A}_n$ (*a*), $\mathbf{B}_n$ (*b*) peptide series: $\mathbf{A_1}$ and $\mathbf{B_1}$ (red), $\mathbf{A_2}$ and $\mathbf{B_2}$ (green), $\mathbf{A_3}$ and $\mathbf{B_3}$ (black), $\mathbf{A_4}$ and $\mathbf{B_4}$ (blue), $\mathbf{A_5}$ and $\mathbf{B_5}$ (magenta). The absorbance was corrected for peptide concentration.

organometallic scaffolds for the construction of foldamers *via* intramolecular H-bonding[6] and as redox probes in the study of ET in polypeptide chains. In fact, self-assembled monolayers (SAMs) of Fc-terminated α-helical peptides have been recently employed as ideal model systems to investigate the ET through peptide chains.[4b,7]

Herein, we describe two series of peptides of different length and rigidity based on the strongly helicogenic α-aminoisobutyric acid (Aib)[8] and containing a terminal Fc unit, Z−$(Aib)_n$−NH−Fc (Z = benzyloxycarbonyl) ($\mathbf{A}_n$, n = 1−5) and Fc−CO−$(Aib)_n$−OMe (OMe, methoxy) ($\mathbf{B}_n$, n = 1−5) (Figure 1). The different orientation of the carbonyl groups in the two helical series generates a macrodipole characterized by an opposite direction with respect to the position of the Fc group. The well-known high chemical stability of Fc in both the neutral and oxidized states can be exploited to inject a positive charge in the peptide chains of the $\mathbf{A}_n$ and $\mathbf{B}_n$ series. The shift and pattern of the vibration of the sensitive spectroscopic tags of peptides, the N−H groups, upon oxidation of the terminal Fc were employed to map the charge delocalization.

Cyclic voltammograms (CVs) of the Fc-peptides $\mathbf{A}_n$ and $\mathbf{B}_n$, recorded under argon in CH_2Cl_2/0.1 M nBu$_4$NPF$_6$, show a single, reversible, one-electron oxidation wave of the Fc unit. As the Aib_n chain grows, the molecules become easier to oxidize in the $\mathbf{A}_n$ series, while they are more difficult to oxidize in the $\mathbf{B}_n$ series (Figure 2). This effect is related to the different orientation of the macrodipole moment of the peptide helix in the two series relative to the position of the Fc group along the chain.[9]

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Table 1. FT-IR Absorption Data (cm^{-1}) of the N−H Stretching Bands of the $\mathbf{A}_n$ Peptide Series

	$\nu(NH_{Fc})$			$\nu(NH_{ureth})$			$\nu(NH_{pept/free})$			$\nu(NH_{pept/H\text{-}bond})$		
	neutral	cation	$\Delta\nu$	neutral	cation	$\Delta\nu$	neutral	cation	$\Delta\nu$	neutral	cation	$\Delta\nu$
$\mathbf{A_1}$	3418	3363	45	3418	3426	−8	-	-	-	-	-	-
$\mathbf{A_2}$	3340	3272, 3230	-	3418	3414	4	3418	3402	16	-	-	-
$\mathbf{A_3}$	3334	3272, 3230	-	3416	3414	2	3416	3402	14	3334	3334	0
$\mathbf{A_4}$	3330	3272, 3230	-	3418	3418	0	3418	3402	16	3330	3322	8
$\mathbf{A_5}$	3324	3272, 3230	-	3418	3418	0	3418	3402	16	3324	3315	9

Table 2. FT-IR Absorption Data (cm^{-1}) of the N−H Stretching Bands of the $\mathbf{B}_n$ Series

	$\nu(NH_{amide/free}$ and $NH_{pept/free})$			$\nu(NH_{pept/H\text{-}bond})$		
	neutral	cation	$\Delta\nu$	neutral	cation	$\Delta\nu$
$\mathbf{B_1}$	~3440	3412	~28	-	-	-
$\mathbf{B_2}$	~3440	3412	~28	-	-	-
$\mathbf{B_3}$	3428	3412	16	3338	3348	−10
$\mathbf{B_4}$	3425	3402	23	3343	3352	− 9
$\mathbf{B_5}$	3422	3402	20	3334	3340	− 6

Information on the intramolecularly H-bonded network of the two series was initially obtained from FT-IR absorption measurements. The N−H stretching (amide A) region allows us to distinguish which amide protons are involved in C═O•••H−N H-bonds and which are not. A band of low intensity at 3400−3450 cm^{-1} is indicative of the occurrence of free N−H groups ($NH_{pept/free}$), while the appearance of a more intense band at 3320−3340 cm^{-1} is related to strongly H-bonded N−H groups ($NH_{pept/H\text{-}bond}$).[10] Formation of intramolecular H-bonds in the C-terminal amide $\mathbf{A}_n$ series starts for peptides containing at least two residues ($\mathbf{A_2}$−$\mathbf{A_5}$), while in the C-terminal ester $\mathbf{B}_n$ series it starts for peptides with at least three residues ($\mathbf{B_3}$−$\mathbf{B_5}$). As the length of the $(Aib)_n$ chain increases, from $\mathbf{A_2}$ to $\mathbf{A_5}$ and from $\mathbf{B_3}$ to $\mathbf{B_5}$, the frequency of the absorption maximum of the $NH_{pept/H\text{-}bond}$ band decreases while its relative intensity increases (Figure 3; Tables 1 and 2). This behavior is indicative of the formation of a progressively more stable and intramolecularly H-bonded 3_{10}-helix structure.[11] Conversely, the frequency of the band corresponding to the two $NH_{pept/free}$ groups is almost constant.

The 3D-structural conclusion was corroborated by a ^1H NMR titration of the NH groups of peptides $\mathbf{A_5}$ and $\mathbf{B_5}$ in $CDCl_3$ solution as a function of added DMSO-d_6, a potent H-bond acceptor. In both cases, only *two* NH proton chemical shifts are sensitive to the addition of the perturbing agent (see Supporting Information). These plots are typical of 3_{10}-helical peptides, as for an α-helical peptide *three* NH protons are expected to be solvated by DMSO-*d*.[8,11]

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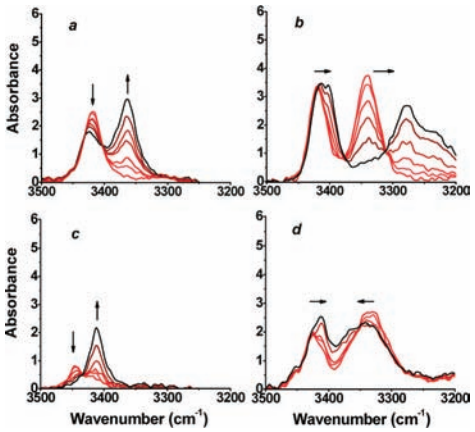

Figure 4. Time evolution of the spectroelectrochemical analyses of the $\mathbf{A_1}$ (*a*) and $\mathbf{A_2}$ (*b*), $\mathbf{B_1}$ (*c*) and $\mathbf{B_3}$ (*d*) peptides in CH_2Cl_2 with 0.1 M nBu$_4$NPF$_6$ as the supporting electrolyte at an applied potential from 0 to 0.4 V (*a* and *b*) and from 0.4 to 0.7 V (*c* and *d*). The absorbance was corrected for peptide concentration.

To study the propagation of the charge along the peptide chain, the FT-IR absorption spectra in the amide A region of the $\mathbf{A}_n^+$ and $\mathbf{B}_n^+$ cations were analyzed and compared to the corresponding spectra of the neutral peptides. Stable solutions of the $\mathbf{A_1}^+$−$\mathbf{A_5}^+$ and $\mathbf{B_1}^+$−$\mathbf{B_5}^+$ cations were obtained by application of the spectroelectrochemistry technique in CH_2Cl_2/0.1 M nBu$_4$NPF$_6$ solution at an applied potential from 0 to 0.4 V and from 0.4 to 0.7 V, respectively. The presence of a positive charge on the Fc moiety of $\mathbf{A_1}^+$ splits the single N−H band into two distinct bands at 3426 and 3363 cm^{-1}, assigned to the urethane N−H group (NH_{ureth}) and to the free amide N−H group directly linked to the Fc^+ moiety (NH_{Fc}), respectively (Figure 4a and Table 1).

In contrast, the oxidation of $\mathbf{A_2}$, in which the first intramolecular H-bond is formed, causes a marked shift to lower energy and the broadening of the N−H absorption at 3363 cm^{-1} which includes two bands (3272 and 3230 cm^{-1}), together with the appearance of two distinct bands at 3414 and 3402 cm^{-1}, assigned to the NH_{ureth} and $NH_{pept/free}$ groups, respectively. The broad absorption at lower energy can be ascribed to the stretching mode of the H-bonded NH_{Fc} considering the high value of the energy shift ($\Delta\nu$ = 45 cm^{-1}) generated by the close proximity to the Fc^+ moiety.

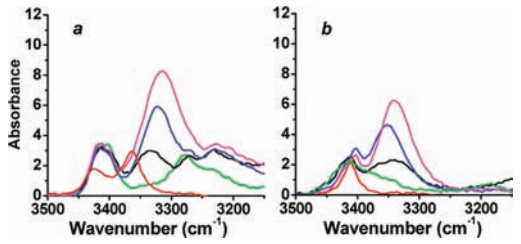

Figure 5. Final spectroelectrochemical FT-IR absorption spectra for the N−H stretching region of cations of the $\mathbf{A}_n$ (*a*) and $\mathbf{B}_n$ (*b*) peptides: $\mathbf{A_1}^+$ and $\mathbf{B_1}^+$ (red), $\mathbf{A_2}^+$ and $\mathbf{B_2}^+$ (green), $\mathbf{A_3}^+$ and $\mathbf{B_3}^+$ (black), $\mathbf{A_4}^+$ and $\mathbf{B_4}^+$ (blue), $\mathbf{A_5}^+$ and $\mathbf{B_5}^+$ (magenta). The absorbance was corrected for peptide concentration.

Looking at the other members of the $\mathbf{A}_n$ series, oxidation of $\mathbf{A_3}$−$\mathbf{A_5}$, similarly to what was observed for $\mathbf{A_2}^+$, leads to the appearance of broad bands at 3272 and 3230 cm^{-1}, assigned to the H-bonded NH_{Fc}, and of the two bands related to the NH_{ureth} and $NH_{pept/free}$ groups (Table 1 and Figure 5a). Interestingly, the presence and the intensity increase of the $NH_{pept/H\text{-}bond}$ band around 3320 cm^{-1} indicate that the intramolecular C═O···H−N H-bonding network is preserved in $\mathbf{A_3}^+$−$\mathbf{A_5}^+$. Moreover, the splitting of the free NH_{ureth} and the $NH_{pept/free}$, even in the longest $\mathbf{A_5}^+$ peptide, strongly suggests that the positive charge is transmitted through the peptide chain to a long distance.

Let us now consider the charge effect in the oxidized $\mathbf{B_1}^+$−$\mathbf{B_5}^+$ peptides. Upon oxidation of $\mathbf{B_1}$, the band related to the Fc^+-linked $NH_{amide/free}$ at ∼3440 cm^{-1} is shifted toward low energy ($\Delta\nu$ = ∼28 cm^{-1}) but to a lower extent with respect to $\mathbf{A_1}$ ($\Delta\nu$ = 45 cm^{-1}) due to the interposition of the carbonyl group between the Fc^+ moiety and the NH group (Figure 4c and Table 2). In the spectrum of $\mathbf{B_2}^+$ the broad absorption is due to the two undistinguished Fc^+-linked $NH_{amide/free}$ and $NH_{pept/free}$ groups. The oxidation of $\mathbf{B_3}$, in which the first intramolecular H-bond is formed (Figure 1), produces a shift of the $NH_{pept/free}$ band at 3428 cm^{-1} toward lower energy ($\Delta\nu$ = 16 cm^{-1}) and of the $NH_{pept/H\text{-}bond}$ band at 3338 cm^{-1} toward higher energy ($\Delta\nu$ = −10 cm^{-1}) (Figure 4d and Table 2).

We explain this opposite oxidation effect on the energy of the H-bonded N−H band in peptides $\mathbf{A_2}$ and $\mathbf{B_3}$ by considering how the positive charge would affect the two different N−H groups. Indeed, the presence of a positive charge on the Fc unit of $\mathbf{A_2}$ is expected to weaken the bond strength of the N−H group directly linked to Fc^+ with generation of a shift to lower energy of the stretching bands. Conversely, the effect of the positive charge in $\mathbf{B_3}^+$ acts on the C═O group directly bonded to the Fc^+ unit, weakening the C═O···H−N H-bond. As a consequence, the N−H bond is reinforced and the corresponding N−H band is slightly shifted to higher energy. Similarly, upon oxidation, the frequencies of the low-energy bands of $\mathbf{B_4}$ and $\mathbf{B_5}$ at 3343 and 3334 cm^{-1}, respectively, related to $NH_{pept/H\text{-}bond}$, are shifted toward higher energy with respect to the neutral peptides ($\Delta\nu$ = −9 and −6 cm^{-1}). In addition, as the peptide chain grows along the series $\mathbf{B_3}^+$−$\mathbf{B_5}^+$, the intensity of this band increases as the number of C═O···H−N H-bonds is enhanced.

In summary, we have synthesized two series of terminally Fc-blocked 3_{10}-helical peptides of varying length and rigidity, and differing in the location of the Fc group relative to the orientation of the macrodipole moment of the peptide chain. We have found that the position of the redox probe has a remarkable consequence on the trends of the oxidation potentials in the two series. As the Aib_n chain grows, an easier or harder oxidation of the Fc moiety occurs in the $\mathbf{A}_n$ and $\mathbf{B}_n$ series, respectively, where the microdipole is oriented away from or toward the organometallic group.

This work represents a rare example of the effect of a positive charge in a peptide chain. It was carried out by comparing the vibrational spectra of the oxidized derivatives with those of their neutral precursors. In particular, this approach has permitted us to demonstrate that the propensity of the Aib residues to form rigid 3_{10}-helices and to promote the onset of intramolecular C═O···H−N H-bonds is maintained even in the presence of an oxidized Fc group. However, the effect of the positive charge on the H-bonding network is opposite in the two series due to the different bridging groups between Fc and the peptide chain (N−H in $\mathbf{A}_n^+$ and C═O in $\mathbf{B}_n^+$). Remarkably, in the $\mathbf{A}_n^+$ series the effect of the charge on the farthest free $NH_{pept/free}$ group is transmitted even for n = 5, which makes this series a valuable tool for mapping the charge distribution in peptide chains.

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Supporting Information Available. Experimental synthetic procedures and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.