Interactions of Ferrocenoyl-Peptides in Solution and on Surfaces

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Summary: The Ferrocenoyl-peptide-cystamines, such as [Fc-Gly-CSA]$_2$ (Gly = glycine, CSA = cystamine), [Fc-Ala-CSA]$_2$ (Ala = alanine) and Fc-conjugates involving collagen models, such as [Fc-(Pro$_2$Gly)$_n$-CSA]$_2$ (Pro = proline, n = 1-6) are readily prepared by solution methods. In solution and the solid state, these systems exhibit intermolecular hydrogen bonding between adjacent peptide chains. For [Fc-Gly-CSA]$_2$ this results in the formation of a supramolecular helicate with two different H-bonding patterns. Ferrocenoyl-collagen-cystamines form assemblies in solution, which melt at elevated tempertures. All systems for monolayers on gold surfaces, which show a well-behaved ferrocene-based electrochemistry, which allows the determination of the spatial requirements of the peptides on the surface.

Keywords: ferrocene; hydrogen bonding; peptide; self-assembled monolayer; supramolecular assembly

Introduction

In solution and in the crystalline state, amino acids and peptides often assemble into extended supramolecular three-dimensional structures. These often form as a consequence of hydrogen bonding between individual molecules.[1] Interestingly, the properties of these peptide supramolecular assemblies are related to the molecular arragement of the subunits. Considerable effort has focussed on the design of secondary structural elements,[2] and on the design of new peptidic materials, such as nanotubes[3] and hydrogels,[4] with potential applications in drug delivery and biomedical engineering. In many cases, scaffolds are used to assist the design and guide formation of a particular peptide structural mimic. Recent effort have been directed at equipping non-covalent supramolecular peptide assemblies with redox-active groups, such as ferrocenes,[5] and give them specific electric properties that may be exploited for biosensing or may have potential for the design of bioelectronic circuitry. Our efforts have been guided by our

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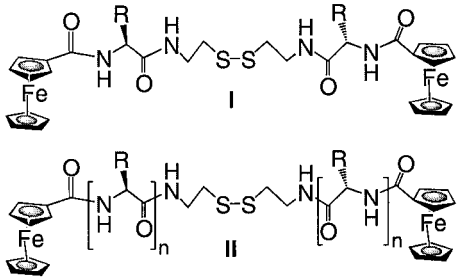
desire to investigate the electron transfer properties in peptides.[6]



For this purpose, we have developed a synthetic pathway allowing the synthesis of ferrocenoyl-labeled amino acid (**I**) and peptide cystamines (**II**), which in turn can be used to prepare ordered two-dimensional arrays on a gold surface.

Hydrogen-Bonding

[Fc-Gly-CSA]$_2$ readily crystallizes from chloroform in the chiral space group $P4_3$. A view of the helical structure is shown in Figure 1.[7]

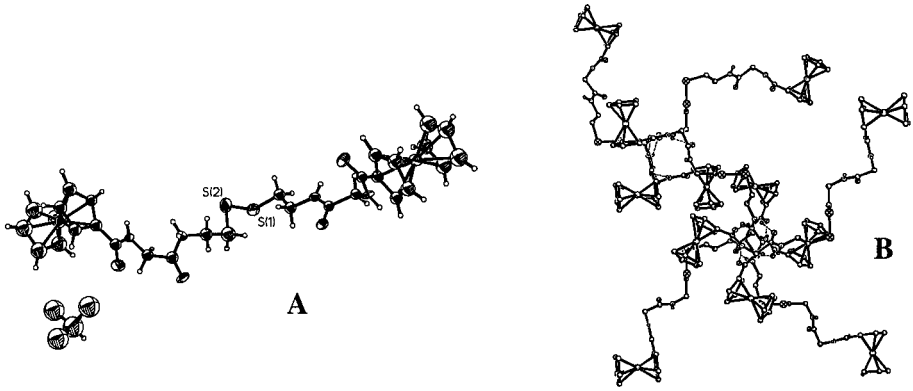


Figure 1. Molecular view [Fc-Gly-CSA]$_2$ (A) of the double helicity in the crystalline state (B) giving a square helix and a twisted helix. The compound crystallizes as a $CHCl_3$ solvate. The solvate does not exhibit any significant interactions with the Fc-peptide.

The structure shows two sets of H-bionding interactions – one which is commonly found in parallel peptide β-sheets and another one which involves a pair of cis-amides. The net result of this complex intermolecular H-bonding interaction is that invividual molecules are forced to turn

with respect to each other, resulting in a helical arrangement. Importantly, the two side of [Fc-Gly-CSA]$_2$ are different and each one is involved in a different supramolecular helical arrangement. The result is an arrangement of two propeller-shaped helices with H-bonded cores linked to each other through a disulphide bridge. The redox active ferrocenoyl moieties are on the outside of a central H-bonded peptide core. Both helices (Figure 1 B) have a pitch height of ca. 14 Å. Although, peptide disulfides often exhibit unusal structural features, the presence of two different chiral helical arrangements in a peptide conjugate is unique and not been described before. Importantly, Fc-amino acid and peptide cystamines exhibit strong H-bonding even in solution. Figure 2 shows the Amide A region of the IR spectrum of [Fc-Ala-CSA]$_2$ in chloroform solution. It clearly shows the presence of two N-H stretching vibrations, typical of H-bonded and non-hydrogen bonded amides. This is readily rationalized by the equilibirium between H-bonded and non-associated molecules.

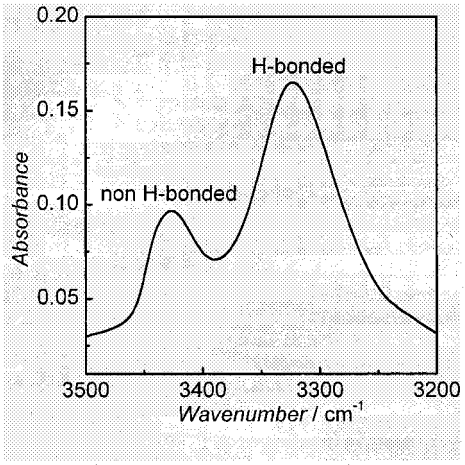


Figure 2. FT-IR absorption bands in the Amide A (NH) region from 3500-3200 cm^{-1} for a 50mM solution of compound [Fc-Ala-CSA]$_2$ in $CHCl_3$ showing hydrogen bonded and non-bonded amide.

Ferrocenoyl-collagen cystamines of the general formula [Fc-(Pro$_2$Gly)$_n$-CSA]$_2$ (n = 1-3) exhibit solution association, typical for collagens. The melting curve for [Fc-(Pro$_2$Gly)$_3$-CSA]$_2$ is shown in Figure 3, having a T_m of 339(2) K. The corresponding „dicollagen" [Fc-(Pro$_2$Gly)$_2$-CSA]$_2$ has

a melting point that is only slightly lower (336(1) K)).

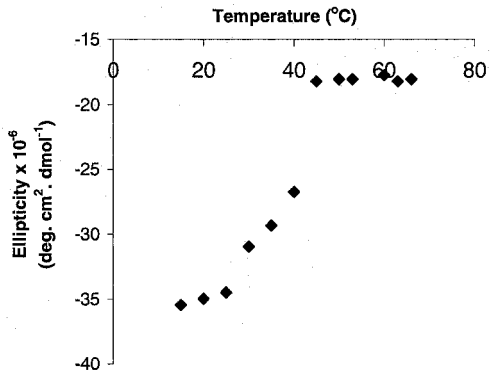


Figure 3. Melting curve for [Fc-($Pro_2Gly)_3$-CSA]$_2$ determined by CD spectroscopy.

Monolayers

Using Fc-amino acid and peptide cystamines we were able to prepare stable and well-behaved monolayers on gold surfaces.[6] The synthetic strategy employed for the synthesis of these systems is shown in Figure 4.

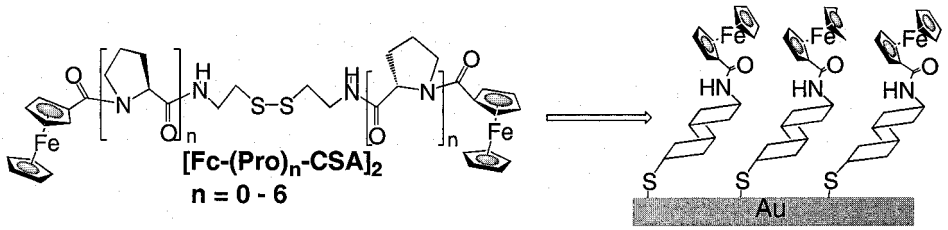


Figure 4. Preparation of monolayers of Fc-peptide cysteamines. Shown is the example of a series of Fc-oligoproline cystamines resulting in stable monolayers.

The redox properties are summaried in Table 1. All monolayers exhibit a single reversible one-electron oxidation. The area occupied by individual Fc-amino acid and peptide molecules on the surface, onbtained from the integration of the oxidative peak currents in the cyclic voltammogram, is given in Table 1. Our results compare well with other helical peptides.[8]

Shorter Fc-peptides which are not able to adopt a helical conformation require less space.

Table 1. Specific Area (in $Å^2$) cccupied by individual Fc-amino acids and peptide cystamines cbtained from the integrated oxidative peak currents.

Entry	Compound	Specific Area
1	$[Fc\text{-}CSA]_2$	85(26)
2	$[Fc\text{-}Gly\text{-}CSA]_2$	70(3)
3	$[Fc\text{-}Ala\text{-}CSA]_2$	81(9)
4	$[Fc\text{-}Pro\text{-}CSA]_2$	123(38)
5	$[Fc\text{-}Ala_2\text{-}CSA]_2$	103(20)
6	$[Fc\text{-}Pro_2\text{-}CSA]_2$	157(20)
7	$[Fc\text{-}Pro_3\text{-}CSA]_2$	180(21)
8	$[Fc\text{-}Pro_4\text{-}CSA]_2$	190(27)
9	$[Fc\text{-}Pro_5\text{-}CSA]_2$	220(40)
10	$[Fc\text{-}Pro_6\text{-}CSA]_2$	240(13)

In general, the oligoproline monolayers are well blocked whereas the other monolayers appear disordered and do not efficiently block the direct electron transfer between the electrode surface and electro active molecules in solution. Thus, there is a fundamental difference between oligoproline monolayers and the other systems, which is most likely related to a better packing on the surface, preventing defects, such as pinholes. Our studies show that H-bonding of the surface supported species is crucial for the formation of a tight monlayer.

Conclusion

Fc-amino acid and peptide cystamines having available amide NH groups assemble to give larger H-bonded assemblies in the solid state and in solution. Expectedly, at higher temperature, these assemblies melt. Monolayers are readily formed. Shorter Fc-amino acid systems tend to produce „leaky“ monolayers which allow access of molecules from the solution to the surface. It is suspected that an increase in the intermolecular H-bonding may result in better blocked surfaces.

Detailed investigations (ellipsometry, RAIRS, AFM) are currently under way to obtain more information about the structural arrangement on the surface.

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