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A novel polynuclear donor complex based on helical peptides with aligned electroactive moieties

Agnieszka Wieckowska [a], Renata Bilewicz [a,*], Aleksandra Misicka [a], Marek Pietraszkiewicz [b], Krzysztof Bajdor [c], Lucjan Piela [a]

[a] *Chemistry Department, Warsaw University, ul. Pasteura 1, 02 093 Warszawa, Poland*
[b] *Institute of Physical Chemistry, Polish Academy of Sciences, ul. Kasprzaka 44/52, 01 224 Warszawa, Poland*
[c] *Industrial Chemistry Research Institute, ul. Rydygiera 8, 01 793 Warsaw, Poland*

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Abstract

Two newly synthesised 21-amino acid peptides substituted with ferrocene groups preserve the helical structure of the peptide and behave as multi-centre donor systems with six electroactive reversible redox centres per molecule. The formal potential of the hexaferrocene compound is slightly less positive than that of its single centre analogue.

1. Introduction

Supramolecular science actually is capable of delivering intelligent advanced materials performing complicated tasks on the molecular level. The interest in multi-centre charge transfer compounds is due to their potential utility as molecular conducting materials, superconductors, efficient catalysts, multielectron reservoirs and molecular memory [1–3] Donors and acceptors can be fixed in space by suitable molecular scaffolding playing mainly a structural role. Porphyrin-laden maquettes [4,5], peptides modified with organic moieties or transition-metal complexes capable of photoinduced electron-transfer within helical domains [6] and peptide-based assemblies of organic chromophores as moieties reminiscent of the reaction centre of photosynthetic bacteria have been reported in the literature. An α-helical peptide – a 21-amino acid peptide composed of 15 L-leucines and six 21-crown-7 L-phenylalanines was found to produce an ion channel from aligned crown ether molecules [7,8]. The peptide backbone seems to be especially useful to control the spatial arrangement of electron donor units. Tetrathiafulvalene derived amino acids and peptides have been described [9,10]. Our aim was to design an electroactive peptide containing a planned number of reversible redox centres located one above each other acting as a well defined polynuclear donor for new donor–acceptor materials, and for the studies of interactions in complex donor–acceptor systems [11].

* Corresponding author. Fax: +48-22-822-5996.
E-mail address: bilewicz@chem.uw.edu.pl (R. Bilewicz).

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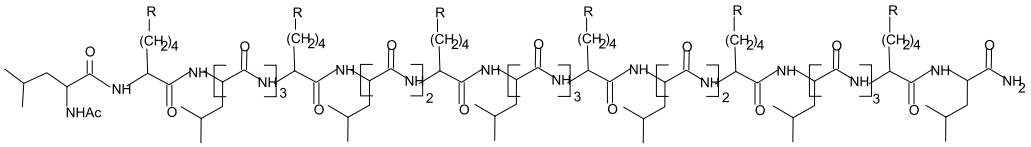
2. Experimental

Formulas of the synthesised 21-oligopeptide and its substituted analogues are shown in Scheme 1. The oligopeptide with the sequence $Ac(Leu–\ Lys–Leu–Leu–Leu–Lys–Leu)_3NH_2$ was prepared by standard solid-phase methods on Rink-resin by using fluorenylmethoxycarbonyl N^{α}-terminal protection and *t*-butyloxycarbonyl N^{ε} protection of lysine. An excess (2 eq) of protected amino acids, 1-hydroxybenzotriazole (HOBt), O-(Benzotriazole-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate and N,N'-diisopropylethylamine (6 eq) was used for coupling reactions, which were monitored by the ninhydrin test. N-terminal amino group of the peptide was acetylated by acetic acid anhydride. After cleavage from the resin with 95% trifluoroacetic acid (TFA) the crude peptide was purified by RP HPLC and characterised by FAB (calc. 2526.5, found 2526.5).

The oligopeptide (compound **1**) in its hexatrifluoroacetate form (m.w. 3210, 68.9 mg) was dissolved in methanol (4 ml) under Ar and stirred with anhydrous Na_2CO_3 (200 mg) for 3 h at room temperature to obtain a free base. After filtering and washing with MeOH (4 ml) the solution was concentrated to 0.5 ml in vacuo, and added to the solution of ferrocenyl aldehyde (65.7 mg, 2-fold molar excess) in MeOH (2 ml) under Ar and reflux. After 10 min the cloudy solution was left to cool to room temperature. The solvent was evaporated to dryness and treated with cyclohexane to remove an excess of ferrocenyl aldehyde. The product (compound **2**)-hexa-Schiff base was filtered and dried in vacuum and the yield was 38 mg. LSIMS MS: $3709.7(M^+)$, $3747.6(M + K^+)$, calcd for $C_{194}H_{290}N_{28}O_{22}Fe_6$: 3701. Compound **1** in its hexatrifluoroacetate form (115.4 mg, 0.036 mmol) was dissolved in a mixture of dry dimethylformamide (1 ml) and acetonitrile (2 ml) under Ar, and 1 ml of *N*-methylmorpholine and 5 mg of 4-N,N-dimethylaminopyridine was added with stirring. 0.5 mmol of ferrocenylcarboxylic acid chloride in 1 ml of acetonitrile (prepared from ferrocenecarboxylic acid and PPh_3/CCl_4 reagent) was added at 0 °C. Stirring was maintained for 3 h at room temperature and the progress of the reaction was monitored on TLC plate (Merck SiO_2). Water was added (10 ml) to quench unreacted ferrocenyl reagent, stirring was continued for 30 min, and the precipitate was filtered, dried in vacuum and suspended in ethyl ether (10 ml) to wash out the remaining triphenylphosphine oxide. The suspension was stirred for 2 h, filtered, washed with ether, and dried under reduced pressure. Yield was 72 mg of compound **3**. MALDI TOF MS: $3821.2(M + Na^+)$, calcd for $C_{194}H_{290}N_{28}\ O_{28}Fe_6$: 3797.7.

3. Results and discussion

The circular dichroism (CD) spectra for unsubstituted peptide **1** and the two electroactive peptides **2** and **3** are shown on Fig. 1. CD curve recorded for unsubstituted starting 21-peptide in



1: R = NH_2

2: R = $FeCp_2CH{=}N{-}$

3: R = $FeCp_2CONH{-}$

Scheme 1. Structures of the compounds studied.

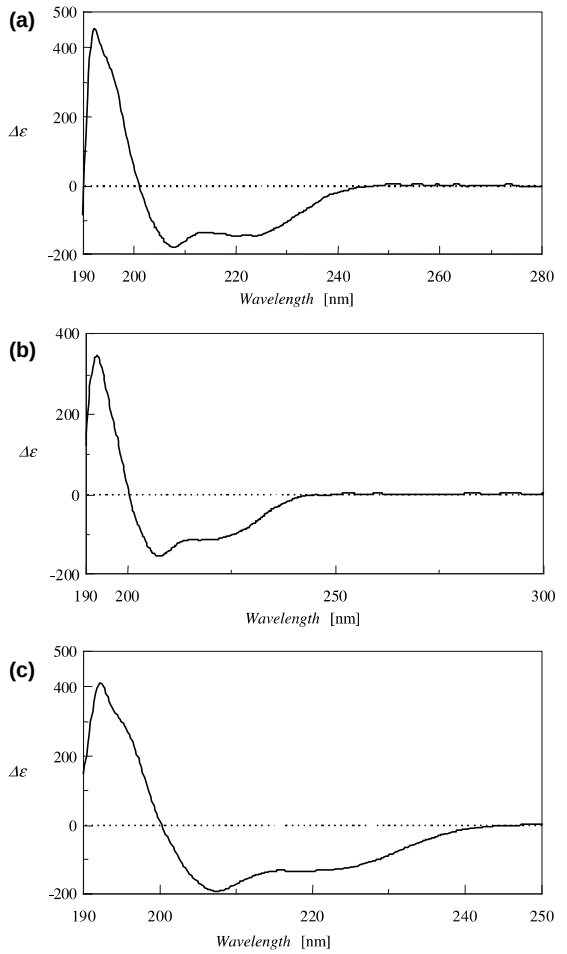


Fig. 1. Circular dichroism spectra of 0.03 mM of the peptide (first from the above) and their hexaferrocene derivatives **2** (0.03 mM) and **3** (0.04 mM) (Schiff base and carboxamide, respectively) recorded in methanol.

methanol at the concentration of 2.75×10^{-5} M showed three CD bands related to Cotton Effect at 220, 208 and 192 nm, with $\Delta\varepsilon = -147$, -178.8 and 452.1, respectively. This indicated the α-helical secondary structure of the oligopeptide. The hexa-Schiff base obtained from 21-peptide and ferrocenylcarboxaldehyde (Compound **2** – Fig. 1b) turned out to be also an α-helical secondary structure. The CD spectrum recorded in 2.68×10^{-5} M solution, showed three CD bands related to Cotton Effect at 208, 218, and 193 nm with $\Delta\varepsilon = -115.5$, -155.9, and +343.4, respectively.

The CD spectrum for hexaferrocenylcarboxamide-based peptide (Compound **3** – Fig. 1c) recorded in 4.13×10^{-5} M solution, showed three CD bands related to 207, 219, and 192 nm with $\Delta\varepsilon = -193.3$, -136.2, and +408, respectively. Thus, the results are consistent with the dominance of helix secondary structure for all three peptides.

In order to check the electron donating ability of all ferrocene systems studied, their formal potentials were obtained from the cyclic voltammetry data. The cyclic voltammograms recorded using a large glassy carbon electrode for 0.1 mM ferrocene and ferrocene peptides **2** and **3** are shown in Fig. 2. The peaks correspond to 1e reversible electrode processes of ferrocene (Fc/Fc^{+}) however, some differences in the peak characteristics are observed upon incorporation of the ferrocene groups into the peptide structure. Namely, a shift of the ferrocene redox potential to more positive values is observed. The formal potentials, E^0, calculated as the midpoint potentials of the oxidation and reduction peak potentials, $(E_{pa} + E_{pc})/2$, are 0.420, 0.536 and 0.593 V for ferrocene, compound **2** and compound 3, respectively. The E^0 differs by ca. 116 mV for compound **2** and 173 mV for compound **3** from that for simple ferrocene. Substitution of a single ferrocene with a $–CH{=}N–C_8H_{17}$ chain [Table 1] results in E^0 equal to 0.550 V hence 130 mV more positive than that of simple ferrocene. Substitution of simple ferrocene with a single $–CONH–C_8H_{17}$ unit gives a larger positive shift of potential, of 177 mV. The latter changes in potential are expected since they follow the general behaviour observed for single ferrocene compounds with electron withdrawing substituents attached directly to the cyclopentadienyl ring. What is, however, interesting, is the positive shift of the formal potential when hexaferrocene compounds **2** and **3** are used instead of simple substituted ferrocenes. This positive potential shift reflects the cooperativity of the centres resulting in higher stability of the system. Thus, the multicentre compound exhibits stronger donor abilities than its single centre analogue. The peak separation $E_{pa} - E_{pc}$ is equal to 64 mV for ferrocene and 74 mV for ferrocene peptides **2** and **3**. The $E_p - E_p/2$ value is equal to 58 mV for ferrocene

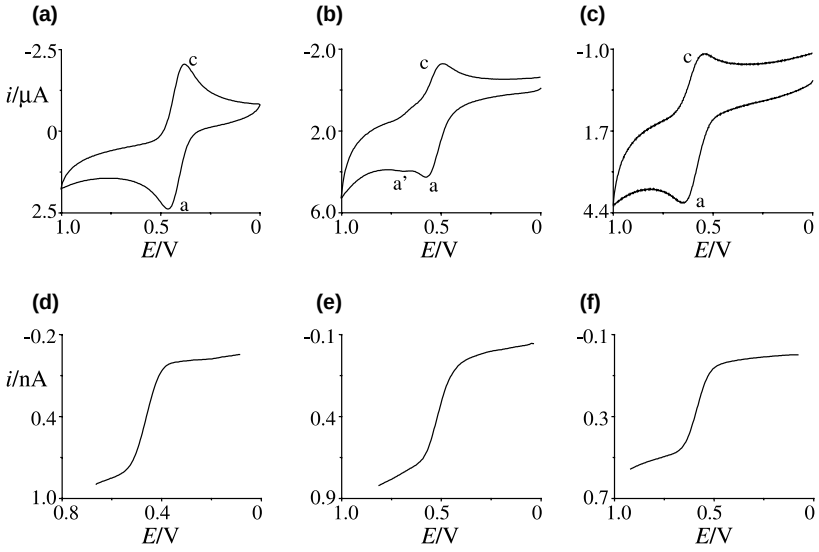


Fig. 2. The cyclic voltammograms for 0.1 mM ferrocene (a, d) and ferrocene peptides **2** (b, e) and **3** (c, f) in 0.1 M TBAP solution of DMF, recorded using (a, b, c) glassy carbon electrode, $r = 1.5$ mm and (d, e, f) microelectrode, $r = 10$ μm. Scan rate: (a, b, c) 0.05; (d, e, f) 0.02 V s^{-1}.

Table 1
Formal potentials of the ferrocene compounds studied

Compound	E^0 (V)	ΔE^{0a} (V)
Ferrocene, Fc	0.420	–
Fc–CH=N–C_8H_{17}	0.550	0.130
Compound **2**	0.536	0.116
Fc–CONH–C_8H_{17}	0.607	0.187
Compound **3**	0.593	0.173

[a] Difference of the formal potentials of the ferrocene derivative and simple ferrocene.

and for the ferrocene peptides it is slightly larger, 72 and 74 mV for the peptides **2** and **3**, respectively. This may also reflect that some communication between the ferrocene centres in the peptides is present and that they do not act as multiple completely non-interacting redox centres [12,13].

A small postpeak sometimes recognized in the case of peptide **2** (peak **a**′) indicated that this peptide was less stable than peptide **3**. Imine bonds present in peptide **2** are known to be less stable than the compounds containing amide bonds.

Since the ferrocene compounds studied revealed close to reversible electrochemical behaviour the number of electroactive centres has been calculated from the voltammetry peak recorded using a large electrode and the plateau current of the stationary currents recorded with a microelectrode (Fig. 2).

Using equations [14]:

$$i_p = 2.69 \times 10^5 A C v^{1/2} n^{3/2} D^{1/2}, \quad (2)$$

$$i_{ss} = 4nFrCD, \quad (3)$$

where A (cm^2) is the electrode area, C (mol cm^{-3}) is concentration, v (V s^{-1}) is scan rate, n is the number of electrons, D (cm^2 s^{-1}) is the diffusion coefficient, F (C mol^{-1}) is Faraday constant, r (cm) is microelectrode radius, i_p (A) is anodic peak current at the large electrode and i_{ss} (A) is the steady state oxidation current recorded using the microelectrode, the diffusion coefficient and the number of centres per molecule were estimated. The D-value was found to be $2.65 \pm 0.21 \times 10^{-6}$ cm^2 s^{-1} and $2.63 \pm 0.26 \times 10^{-6}$ cm^2 s^{-1} for **2** and **3**, respectively, from three experiments in different solutions of the peptide. Best solubility of the peptides was found in DMF, however for more diluted solutions also acetonitrile was suitable as the solvent. The concentration of centres calcu-

lated using Eqs. (2) and (3) was $6.14 \pm 0.24 \times 10^{-7}$ mol cm^{-3}, when 1×10^{-7} mol cm^{-3} of peptide was used, which meant that all six centres were active in the molecule of the ferrocene peptide **2**. In case of compound **3** this value is $6.25 \pm 0.24 \times 10^{-7}$ mol cm^{-3}. Chronoamperometry performed at potential 0.7 V hence more positive than the oxidation of ferrocene groups in the peptides confirmed the diffusion coefficient values and the activity of all six redox-centres.

The UV–VIS electronic spectra for ferrocene, compound **2** and **3** in methanol solutions with and without the presence of electron acceptor (chloranil) are shown in Fig. 3. In the spectrum of ferrocene an absorption band occurs in the visible region at 445 nm (Fig. 3a). The methanol solutions of compound **2** and **3** exhibited similar spectra in the visible region with the absorption band for ferrocene chromophore occurring at 455 and 430 nm, respectively (Fig. 3b,c). Mixing of deoxygenated ferrocene and chloranil solutions did not produce any changes in the absorption spectrum above 500 nm (Fig. 3d) suggesting the lack of substantial donor–acceptor interactions. The absorption bands characteristic for ferrocene and chloranil chromophores are marked with arrows in Fig. 3d. On the other hand, mixing of deoxygenated solutions of chloranil and compound **2** in methanol, lead to the formation of a new broad band at ca. 680 nm (Fig. 3e). The final absorbance of this band (after adding six molar equivalents of chloranil) was about three times lower than that of the ferrocene chromophore present in compound **2**. The latter was shifted with increasing chloranil concentration towards longer wavelengths and finally appeared at 470 nm. However, similar experiments with compound **3** mixed with chloranil (Fig. 3c,f) did not produce any significant spectral changes. Due to a negative result of the experiment with compound **3**, a further experiment including a substituted ferrocene containing the imino group (substituent = –CH=N–C_8H_{17}) and chloranil solutions was performed as well. The observed spectral changes were similar as for the compound **2**, suggesting that their origin was rather related to the presence of the imine bond. As pointed above, the formal potentials of the ferrocene compounds containing the imine group were more negative than those of the corresponding amide group compounds, hence stronger activity towards acceptors in the solution could be expected for the former.

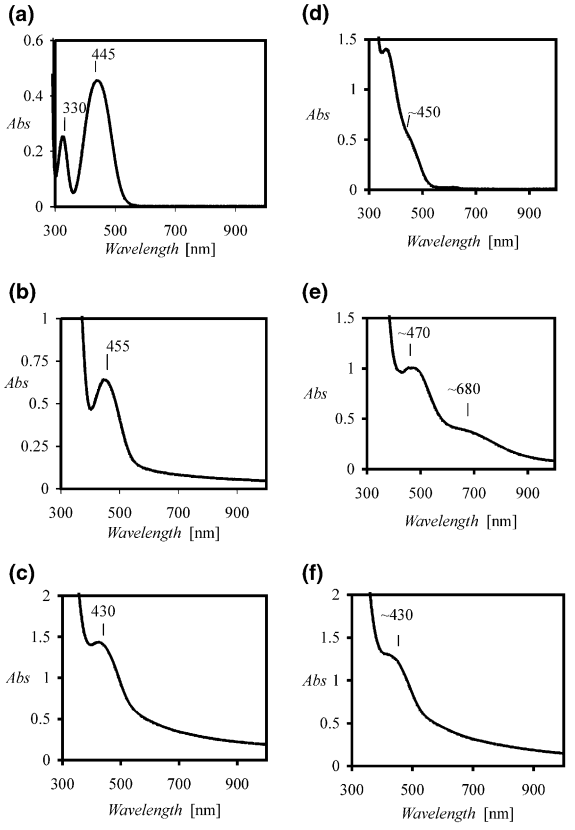


Fig. 3. UV–VIS absorption spectra for: (a, d) 5 mM ferrocene and (b, e) 0.4 mM compound **2** and (c, f) 0.3 mM compound **3** in methanol solution in the absence and presence of 2–5 mM chloranil.

Electrochemical studies of peptides in solutions containing electron acceptors confirmed the different behaviour of electroactive peptides **2** and **3** in the presence of chloranil or quinone. A new anodic signal **a**′ (Fig. 2b) appeared and increased at the expense of the original oxidation peak, **a**, upon increasing the concentration of these acceptors in the solution of compound **2**. This indicates that a reaction takes place between peptide **2** and acceptors such as chloranil and quinone. On the other hand, nothing is changed in the voltammogram of compound **3** upon addition of these

acceptors. Lack of interactions with the amide-bonded electroactive peptide indicates that not the ferrocene donor group itself but the imine moiety together with ferrocene is the source of interaction with the acceptors observed in case of compound **2**. Therefore, compound **3** with ferrocenes attached via amide bonds is favourable if the peptide is to be used as multi-centre outer sphere electron donor system while increase of its donor abilities can be obtained by increasing the number of electroactive centres in the polypeptide backbone. Studies in this direction are continued in our laboratory.

4. Conclusions

Novel well-defined 21-amino acid peptides substituted with six independent electroactive ferrocene groups were synthesized and the helical structures of these new electroactive peptides were proved by CD measurments. The number of electroactive centres and diffusion coefficients for both peptides were determined using two independent electrochemical methods: chronoamperometry and voltammetry. The imine moiety in the peptide is reacting chemically with acceptors added to the solution which precludes its application for the studies of D–A interactions. The electroactive peptide not possessing an imine bond is a good candidate for further studies of donor–acceptor interactions in complex systems.

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