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Electron transfer through organic monolayer films with oligoglycine spacers

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Abstract

Self-assembled monolayers were designed as model systems for the investigation of electron transfer mediated by peptide chains. A thiol derivative containing a cysteamine linker, four glycine residues and a ferrocene head group—Fc-(CO)-$(Gly)_4$-NH-$(CH_2)_2$-SH, was synthesized and used for preparation of monolayer assemblies. Electrochemical methods were employed to measure the electron transfer rates through one-component oligoglycine monolayers and through mixed monolayers with oligoglycine molecules (0.2–0.5%) embedded in the alkanethiol matrix. The standard rate constant for electron tunneling through the pure oligolglycine monolayer ($821 \pm 78\ s^{-1}$) is larger than that through the mixed monolayer with alkanethiols as the main component ($36 \pm 7\ s^{-1}$) and larger than for the corresponding alkanethiol monolayer ($12\ s^{-1}$).

Keywords: Chemically modified electrodes; Monolayers; Peptide; Electron transfer

1. Introduction

Bridge-mediated electron transfer is one of the crucial elements in many biological processes like photosynthesis and cellular respiration. In biological systems, single electron transfer occurs rapidly from donor to acceptor over long distances and the efficiency of this process is dictated by the strength of the electronic coupling between redox sites, accordingly to the relation [1]:

$$k_{ET} = \left(\frac{2\pi}{\hbar}\right)|H_{AB}|^2(FC) \quad (1)$$

where k_{ET} is the rate constant of electron transfer, H_{AB} describes the electronic coupling between the donor and the acceptor, (FC) is the Franck-Condon factor. The electronic coupling element is strongly affected by the length and chemical nature of the bridge separating the redox sites. Therefore, in the studies of long range electron transfer, one of the most important issues is how the rate of electron tunneling through the organic bridge is influenced by the structure of the medium separating donor and acceptor [2–5].

It has been proven that self-assembled monolayers (SAMs) of thiol derivatives are suitable to investigate the kinetics of mediated electron transfer [6,7] and the electronic coupling through the bridge can be modulated by the introduction of chemical modification into the molecules forming the monolayer [8–19]. The structural and chemical properties of SAMs make them suitable to modify the electrode surface in a predictable way and the electrochemistry is extensively used to investigate the kinetics of electron transfer through these systems.

We have recently been involved in studies of non-electroactive and electroactive alkanethiol SAMs modified by replacing methylene units in the alkane chain by an amide moiety [13–16]. We found that isolated amide groups do not affect the electron transfer kinetics while hydrogen bonded amides contribute to the overall electron transport though the monolayer. This leads to the suggestion that hydrogen bonds in the monolayer may increase the chain–to–chain coupling more efficiently than simple van der Waals interactions.

High efficiencies of electron transport have been reported recently by Galka and Kraatz for one-component monolayers of oligoproline derivatives [18]. These

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interesting observations led us to synthesize a new group of thiol compounds with molecular bridges more relevant to biological units—the electroactive polypeptides. Here, we report two types of SAMs based on the thiol derivative of oligoglycine. The first, is a one-component monolayer of electroactive oligoglycine with a cysteamine linker, Fc-(CO)-$(Gly)_4$-NH-$(CH_2)_2$-SH (Fig. 1). The second type of SAM is a mixed, two-component system of the same redox-active oligoglycine introduced into the *n*-dodecanethiol ($C_{12}SH$) matrix. The kinetics of electron transport through these two types of monolayers were found to be very different and in both cases faster than through a simple alkanethiol monolayer of similar length. This was ascribed to the differences in the structure of these monolayers and in the presence of water molecules inside the layer.

2. Experimental

2.1. Chemicals

The synthesis of Fc-(CO)-$(Gly)_4$-NH-$(CH_2)_2$-SH was performed by solid phase synthetic techniques starting from 0.3 mmol g^{-1} substituted cysteamine 4 methoxytrityl resin (Novabiochem). Successively four 9-fluorenylmethoxycarbonyl (Fmoc) glycines and ferrocenecarboxylic acid (Fc-COOH) were coupled using 1,3-diisopropylcarbodiimide (DIC) as the coupling reagent and monitored by a qualitative ninhydrin test. The cleaveage was performed by 5% trifluoroacetic acid (TFA) in dichloromethane (DCM) containing 5% triisopropylsilane (TIS). Purification of the final compound was accomplished by preparative RP-HPLC and analysed by ESI-MS (calc/found) (517/517).

Dodecanethiol was purchased from Aldrich and it was used as received. Perchloric acid was purchased from POCh Gliwice. Water was distilled and passed through a Milli-Q purification system. The final resistivity of water was 18.2 MΩ cm^{-1}.

2.2. Preparation of monolayer modified electrodes

The monolayers were self-assembled on Au(1 1 1) substrates (Arrande). The substrates were 200–300 nm thick gold films evaporated onto borosilicate glass precoated with a 1–4 nm adhesion layer of Cr. Before adsorption of the monolayer, the substrates were etched for 10 min in hot nitric acid and then flame annealed in a Bunsen burner. Self-assembly was carried out from 1 mM ethanolic solutions of the oligoglycine derivative. In the case of mixed monolayers, the solution contained alkanethiol and oligoglycine derivative. To avoid possible phase separation of the components within the mixed monolayer, the mole fraction of the redox-active component in solution was below 1% and the deposition was carried out at 40 °C [20,21]. After 4 h of being soaked, the substrates were rinsed with ethanol and water. Fresh samples were prepared before each experiment.

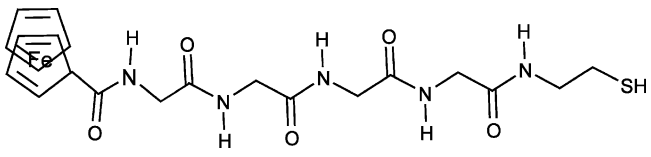

Fig. 1. Structure of the peptide studied.

2.3. Instrumentation

STM measurements were performed using a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) equipped with a low current converter. The images were taken under ambient conditions using commercially available Pt–Ir tips.

Electrochemical experiments were carried out with an Autolab PGSTAT30 electrochemical analyzer, in a three-electrode system with Ag wire as a reference electrode, platinum foil as a counter electrode and the monolayer covered gold substrate as a working electrode. The supporting electrolyte was 1 M $HClO_4$. All measurements were performed at 25 °C.

3. Results and discussion

Single component oligoglycine and the same mixed with dodecanethiol monolayers were characterized by electrochemical methods (cyclic voltammetry and impedance spectroscopy). The surface coverage of oligoglycine molecules was estimated from the area of the cyclic voltammetric peaks corresponding to the oxidation of ferrocene centers [22,23].

The capacitance of the electrode modified with a one component monolayer of oligolglycine derivative was 11.7 ± 3.3 μF cm^{-1}, hence, larger than for simple alkanethiol monolayers. This is due to the higher polarity of the peptide molecules compared to simple alkanethiols. Water molecules can also reside inside the monolayer close to the peptide bonds in contrast to the situation in hydrophobic alkanethiol monolayers.

The cyclic voltammogram recorded for the one-component monolayer of oligoglycine derivative shows a close to reversible system of peaks (Fig. 2A) but with the half-height width of the peaks larger than the 90.6 mV expected for the ideal 1e process [23]. The increased width of the peaks reflects the presence of interactions between the ferrocene headgroups. The area occupied by one molecule calculated from the charge of the peak is 34 ± 5 $Å^2$, indicating that the ferrocene moieties are densely packed and close to each other. The ferrocene molecular area is about 34 $Å^2$ [24], hence monolayer

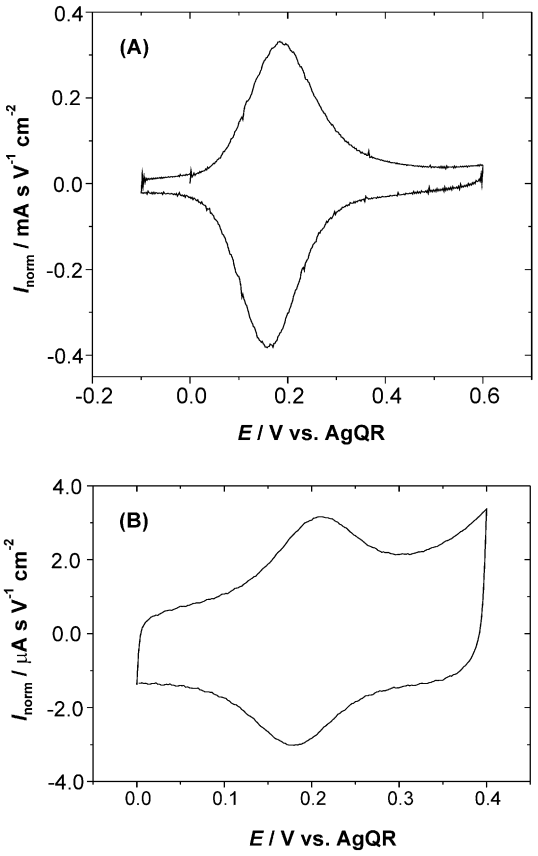

Fig. 2. Cyclic voltammograms recorded in 1 M $HClO_4$ at the electrode covered with (A) a one-component monolayer of oligolglycine derivative and (B) a mixed SAM of dodecanethiol and oligoglycine.

packing seems to be determined by the size of this headgroup. The presence of interactions between the redox groups may affect the electron transfer efficiency through the monolayers, therefore, in order to minimize these interactions we prepared a different type of monolayer—a mixed monolayer of Fc-(CO)-$(Gly)_4$-NH-$(CH_2)_2$-SH and $C_{12}SH$ in approximately 1:99 ratio. The structure of the monolayer is shown schematically in Fig. 3.

The STM data presented in Fig. 4 clearly show that the self-assembly procedure described in the Section 2 leads to a random distribution of oligoglycine derivative within the dodecanethiolate monolayer. Features due to the oligoglycine appear as protrusions (bright spots). This corresponds to the situation when the molecules of oligoglycine are extended above the $C_{12}SH$ film. This is expected since the molecules of oligoglycine are longer than the $C_{12}SH$ and the physical height difference between the oligoglycine molecules and the $C_{12}SH$ matrix is about 8 Å (assuming a 30° tilt angle). The STM topographic height difference between the oligoglycine molecules and the top of the film was found to be 3–5 Å, which is less than the physical height difference. This result indicates that the transconductance for oligoglycine is lower than for $C_{12}SH$, which is

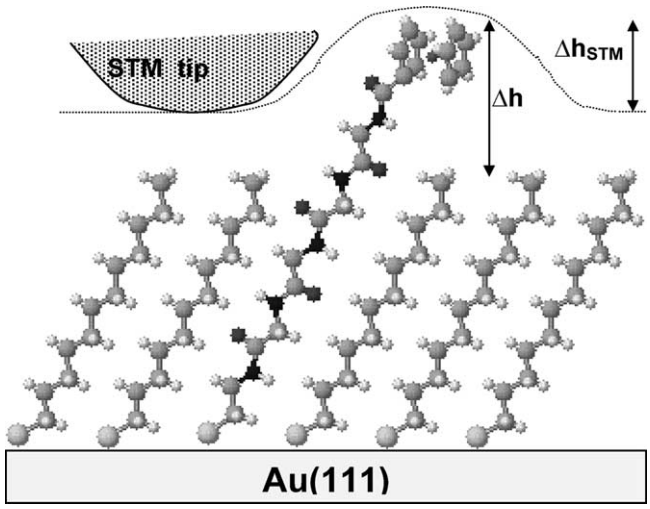

Fig. 3. A schematic representation of the mixed monolayer and the trajectory of the STM tip. During the STM imaging, the position of the tip depends on the transconductance of the molecules forming the mixed monolayer.

reasonable if we consider again the difference in length of these molecules. The sizes of the features ascribed to the oligoglycine are larger than expected for single molecules. This is reasonable because single molecules are much sharper than the STM tip (Fig. 3), and as a result, the protruding molecule of oligoglycine 'images' the tip structure [25,26]. On the basis of the STM data described above we conclude that molecules of oligoglycine were successfully introduced into the alkanethiolate monolayer.

Fig. 2B shows the typical cyclic voltammogram obtained for a mixed monolayer. Now the half-width of the peak is close to that predicted for an ideal case with no intermolecular interactions. The fraction of electroactive molecules incorporated into the organothiol matrix was in the range 0.2–0.5%, which was small but still much higher than the surface coverage estimated from the STM measurements (Fig. 4). This suggests that a large number of ferrocene centers is located closer to the external plane of the monolayer defined by the terminal groups of the dodecanethiol diluent since, in such a case, the difference in the height between the electroactive and non-electroactive molecules is too small to be 'visible' in the STM image. The different location of ferrocene units can be connected with either gauche defects or cis–trans isomerization which can take place in the oligoglycine chain. Differences in the secondary structure between oligoglycine molecules may also change the position of ferrocene moieties relative to the monolayer surface.

The capacitance of the mixed monolayer in the double layer region is approximately 2 μF cm^{-2}. Although the percentage of oligoglycine molecules within the monolayer was small, the capacitance is slightly increased compared to the value 1.4 μF cm^{-2} for the pure $C_{12}SH$

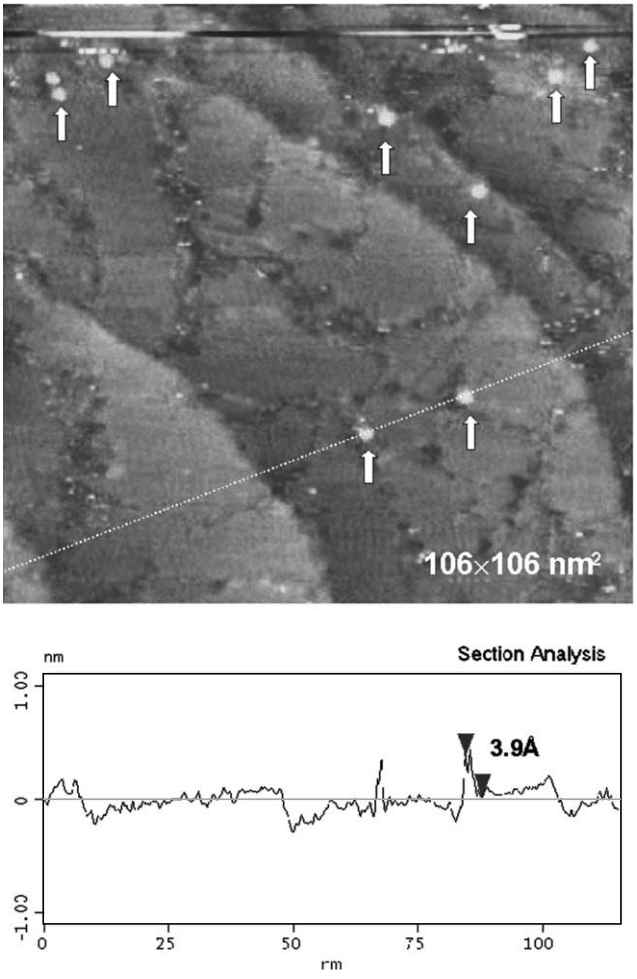

Fig. 4. STM image of dodecanethiolate monolayer on Au(1 1 1) with inserted molecules of electroactive oligoglycine (indicated by white arrows). The image was taken at ambient conditions, tunneling current = 1 pA, tip bias = 1000 mV. Cross-section profile (along the white line) shows the STM height difference between the oligoglycine molecules and top of the dodecanethiolate film.

monolayer. This small difference is reasonable since we introduced relatively polar molecules into the assembly.

The rate constants for electron transfer through both types of monolayers were obtained using impedance techniques [27–29]. Data analysis involved application of a Randles equivalent circuit, which is appropriate for modeling of a redox-active film adsorbed on an electrode surface [27–29]. The complex plane impedance plot and the equivalent circuit are shown in Fig. 5. The equivalent circuit consists of a solution resistance R_S, charge-transfer resistance R_{CT}, double-layer capacitance C_{DL} and adsorption pseudocapacitance C_{AD}. The magnitudes of fitted circuit elements were used to calculate the rate constants. The potential applied to the electrode was the formal potential for the Fc/Fc^+ redox couple. Under these conditions the electron transfer rate can be determined using the following relations [30,31]:

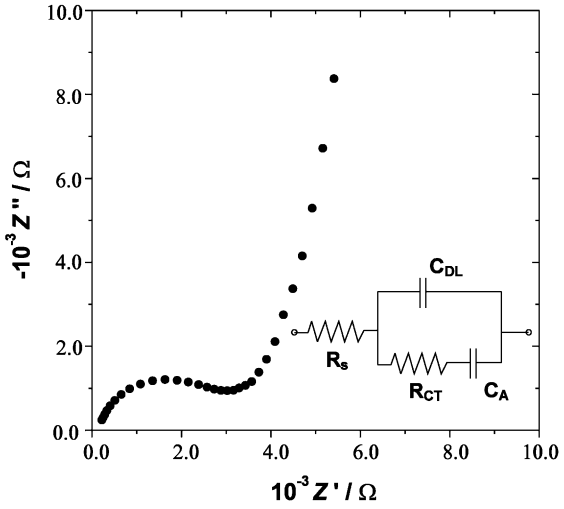

Fig. 5. Complex plane impedance plot obtained for mixed SAM at the formal potential for the Fc/Fc^+ couple. Points acquired between 1.78 Hz and 10 kHz are shown.

$$C_{AD} = \frac{F^2 A \Gamma}{4RT} \tag{2}$$

$$R_{CT} = \frac{2RT}{F^2 A \Gamma k_{ET}} \tag{3}$$

where Γ is the coverage of electroactive species per unit area and A is the area of the electrode. Using Eqs. (2) and (3), the electron transfer rate k_{ET} can be obtained from the equation:

$$k_{ET} = \frac{1}{2R_{CT}C_{AD}} \tag{4}$$

The rate constant k_{ET} corresponds to the standard rate constant, measured at the formal potential of redox couple.

The standard rate constant for electron transfer through the oligoglycine spacer was found to be $k^{0\prime} = 36 \pm 7\ s^{-1}$. This value is slightly higher than expected on the basis of previously reported results for non-hydrogen-bonded-amide containing monolayers [13] and for monolayers of simple *n*-alkanethiols of the same number of atoms in the chain [19,32]. It was found that the electronic coupling through the non-hydrogen-bonded amide moiety is similar to that observed for two methylene units. Therefore, the rate constant measured for the oligoglycine spacer should be comparable with the values obtained for a simple alkyl chain of the same length. Assuming that the tunneling coefficient $\beta = 1.0\ Å^{-1}$, the rate constant should be about 12 s^{-1} [19,32]. The difference can be caused by several factors. The most obvious one is the presence of defect sites in the monolayer. When the electroactive molecules are adsorbed at the defect sites, the ferrocene moiety can be located within the monolayer and direct electron transfer between the redox site and the electrode surface is

possible. If a large fraction of electroactive molecules is adsorbed at the defect sites, the rate constant for electron transfer would be higher than expected. In order to exclude this possibility, we performed an additional control experiment. In this experiment, first we prepared a one-component monolayer of dodecanethiol, and then the electrode was incubated in a solution of 1 mM ferrocene-modified oligoglycine. Under these conditions, some portion of dodecanethiol molecules forming the monolayer is exchanged with the electroactive component. The molecules adsorbed at the step edges and grain boundaries exchange faster than those adsorbed at the terrace sites [24,33]. This means that the large fraction of electroactive molecules is located at the defect sites, thus the standard rate constants measured for this system should reflect disorder of oligoglycine molecules and random location of the redox site with respect to the electrode surface. The standard rate constants obtained from these experiments were in the range of 150–300 s^{-1}, and the results were not reproducible. It is clear that the rate constant obtained for monolayers prepared by co-adsorption of molecules ($k^{0\prime} = 36 \pm 7\ s^{-1}$) is much less affected by the presence of defect sites and we can assume that the main contribution to the overall rate constant is from the through-chain coupling.

The standard rate constant for electron transfer through the one-component monolayer containing only oligoglycine spacers was found to be $k^{0\prime} = 821 \pm 78\ s^{-1}$. The large difference of the rate constants in the case of one-component and mixed monolayers can be understood by considering the differences in the structures of these monolayers and the nature of the chain-to-chain coupling.

It should be noticed that both values of the rate constant are higher than those obtained for simple *n*-alkanethiols of similar length. More efficient electron tunneling through the peptide chain can result from the nature of the bridge. This assumption is reasonable, because *ab initio* calculations of the electronic coupling for the polyene and polyamide bridges confirm the experimental trends. It follows from the simplest UHF 3-21G//UHF 3-21G calculations on the lowest triplet states of diradicals $(CH_2)_{13}$ and $CH_2(NHCOCH_2)_4$ that the coupling for the radical cation increases from 336 to 4738 cm^{-1}, while for the radical anion it changes from 70 to 3881 cm^{-1}. Obviously, care should be taken when considering these numbers since the couplings quoted above were obtained from simple UHF calculations on diradicals followed by an application of the Koopmans theorem [34]. This very approximate treatment neglects the orbital relaxation, so its accuracy is very limited. In addition, the geometries of the diradicals were fixed at their equilibrium values, and do not correspond to the crossing of the diabatic potential energy surfaces. However, assuming that the error in the calculations is of the order of 50%, theory still predicts a noticeable change in the electronic coupling through the bridges constructed from amide units.

4. Conclusions

Higher rate constants obtained for monolayers containing polypeptide spacers may be explained in a number of ways. The *ab initio* calculations of the electronic coupling for the polyene and polyamide bridges indicate that more efficient electron tunneling through the peptide chain can result from the nature of the bridge. In the monolayer, the presence of surrounding molecules has to be taken into account when the electron transfer efficiency through the monolayer is considered. For example, Finklea et al. found that the electron transfer rate through electroactive alkanethiol molecules can be increased due to conformational changes of the chain to make contact between the redox center and the terminal groups of a nearby 'diluent' thiol [35]. We found that part of oligolglycine molecules in our monolayer assembly was not detected in the STM images indicating that they may not be rigid enough in this environment to extend beyond the alkanethiol matrix. These 'defective' molecules may have redox centers located differently relative to the monolayer surface due to conformational changes, *cis–trans* isomerization in the chain, gauche defects, different secondary structure, etc. [36]. We can assume that only the well organized part of the redox-active molecules, rigid and protruding above the surface defined by the alkanethiol 'diluent' contributes to the observed increased electron transfer efficiency through the mixed monolayer containing peptide chains. This is reasonable, if we consider the fact that disordered chains usually contribute less to the overall coupling [37,38]. Moreover, the pathway through the diluent thiol involves electron tunneling through van der Waals contact between the redox site and the terminal methyl groups of dodecanethiol. In this case the electron tunneling probability is several times lower than for through bonds [39]. Therefore, the contribution of this pathway to the overall coupling is small.[1]

[1] In order to verify the assumption that the current is controlled by tunneling through the peptide chain, we also did a complementary experiment, where the tetradecanethiol was used as a diluent. In this case, the longer chain preserves the conformational changes of electroactive peptide, thus the ferrocene moiety is located above the external plane of the monolayer defined by the terminal groups of the diluent thiol. The average value of standard the rate constants for such a system was $k^{0\prime} = 30\ s^{-1}$. This confirms that the current is controlled by the transport of electrons through the peptide molecules and not through the diluent thiol.

Investigations of the electron transfer mediated by polypeptides for a wide range of lengths of the bridge should allow a more detailed study of the contributions of various electron pathways to the overall electron transfer efficiency through the monolayers. This aspect and a more systematic theoretical study of the coupling in the radical cationic and anionic species near the crossing of the diabatic surfaces will be reported in a forthcoming paper.

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