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Vectorial Electron Transport at a Long Distance by Helical Peptides

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We have demonstrated that α-helical peptides mediate electrons at a long distance over 4 nm, and this electron mediating property is explained by the incoherent hopping mechanism. Further, the electron transfer from *C* terminal to *N* terminal along the helix peptide is faster than that of the opposite direction. This rate enhancement is due to the large dipole moment generated along the helical peptide. The helical peptide is a good electron mediator as a long molecular wire notably with a specific molecular diode property.

Key words: electron transport, helical peptide, self-assembled monolayer, molecular electronics, molecular diode

1. INTRODUCTION

There are several electron transfer mechanisms for molecular wires: coherent electron transfers of nonresonant type and on resonance, incoherent electron transfer, and quasiparticles [1]. Contribution of the incoherent conductivity appears to be predominant for longer molecular wires because of its Ohmic behavior. We have demonstrated that α-helical peptides mediate electrons at a long distance over 4 nm, and this electron mediating property is explained by the incoherent hopping mechanism [2]. Further, the electron transfer from *C* terminal to *N* terminal along the helix peptide is faster than that of the opposite direction. This rate enhancement should be due to the large dipole moment generated along the helical peptide [3,4]. The dipole moment also affects the potential of the redox group when it is attached to the end of the helix peptide [5]. A self-assembled monolayer composed of two helix peptides, one with a ruthenium compound and being immobilized via *C* terminal and the other with an *N*-ethylcarbazole group and being immobilized via *N* terminal, was prepared. The direction of photocurrent generation is shown to be opposite by switching the excitation wavelength from one sensitizer to the other [6]. Photocurrent along the dipole moment is promoted. Taken together, the helical polypeptide is a good electron mediator as a long molecular wire notably with a specific molecular diode property.

The standard electron transfer rates of the 16- and 17-residue helical peptides were, however, found to be very low. The electron transfer at the interface between gold and the peptides should be suppressed to decrease the overall transfer rates. In order to clarify this point, a new helical peptide was here synthesized, which comprises a phenyl group at the N-terminal as a linker between gold and the peptide (AcSL16Fc; Fig. 1). π-Electrons of the phenyl group should promote the electron transfer at the interface. The effect of the aromatic group was evaluated by taking the peptide carrying a methylene linker at the N-terminal as reference (SSL16Fc; Fig. 1). Further, another new helical peptide, which possesses two ferrocene units in the middle of the 16-residue peptide and at the N-terminal, was synthesized (Fcl8FcL8SS; Fig. 1). The inserted ferrocene unit is expected to relay the electron transfer through the peptide. To examine this effect, the electron transfer rate of Fcl8FcL8SS was compared with that of the peptide carrying one ferrocene unit at the N-terminal (FcL16SS; Fig. 1).

2. EXPERIMENTAL

The molecular structures of the helical peptides and the synthetic schemes of Fcl8FcL8SS and AcSL16Fc are shown in Fig. 1. The peptides were synthesized by the conventional liquid phase method as follows.

Fcl8FcL8SS

N-(1'-(*N'*-(*N''*-*tert*-Butyloxycarbonylethylenediamine))carbonylferrocenecarbonyl)-L-alanine benzoate (BFcB), Boc-(L-Leu-Aib)$_4$-OBzl (BL8B) and Boc-(D-Leu-Aib)$_4$-OBzl (Bl8B) were prepared according to the literature [2,7]. The abbreviations, Boc, Leu, Aib and OBzl represent *tert*-butyloxycarbonyl, leucine, α-aminoisobutyric acid and benzyl ester, respectively. The benzyl ester of BFcB was removed by catalytic hydrogenation in dichloromethane with 10 wt% palladium carbon to afford BFcOH. According to the previously reported method [2], the C-terminal of BL8B was deprotected and reacted with 1,2-dithia-3-(1-amino-*n*-pentyl)-cyclohexane hydrochloride to afford BL8SS. Boc groups of Bl8B and BL8SS were removed by the treatment with 4N HCl in dioxane. The respective peptides were reacted with BFcOH to afford BFcl8B and BFcL8SS, respectively. The C-terminal of BFcl8B (15 mg) and the N-terminal of BFcL8SS (17 mg) was deprotected to afford BFcl8OH and HFcL8SS, respectively. Then, the prepared BFcl8OH and HFcL8SS were coupled in chloroform (2.0 ml) in the presence of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 10 mg)

and *N,N*-diisopropylethylamine (DIEA, 9.0 μl) under N_2 atmosphere at 0 °C for 10 min and thereafter at room temperature for 24 h. The solvent was evaporated, and the crude product was purified by a Sephadex LH20 column (methanol as eluant) and washed with *n*-hexane to afford an orange powder (11 mg, 36 %). TLC: R_f (chloroform/methanol/acetic acid = 95/5/3 v/v/v) = 0.26, R_f (chloroform/methanol = 10/1 v/v) = 0.45. ^{1}H NMR (400 MHz, CD_3Cl): δ(ppm) 0.85 (48H, LeuCδH_3), 1.36-1.90 (96H, $(CH_3)_3$C, AlaCβH_3, LeuCβH_2, LeuCγH, AibCβH_3, $NHCH_2(CH_2)_4CHCH_2CH_2SS$, $NHCH_2(CH_2)_4CHCH_2CH_2SS$), 2.40 (1H, m, $NHCH_2(CH_2)_4CHCH_2CH_2SS$), 2,91, 3.17, 3.52 (4H, brm, 8H, $NH(CH_2)_2NH$), 3.12 (4H, $NHCH_2(CH_2)_4CHCH_2CH_2SS$), 3.48 (1H, m, $NHCH_2(CH_2)_4CHCH_2CH_2SS$), 3.80-4.16 (10H, AlaCα$H$, LeuCα$H$), 4.23-5.40 (16H, ferrocenyl-H), 6.50, 7.35-8.25 (23H, amide-H). MS (FAB, matrix; nitrobenzylalcohol): m/z 2638 (calcd for $C_{127}H_{207}Fe_2N_{23}NaO_{24}S_2$ $[(M+Na)^+]$ m/z 2638.4).

AcSL16Fc

BFcB was treated with 4N HCl in dioxane to afford HFcB. Boc-(L-Leu-Aib)$_8$-OBzl (BL16B) was synthesized according to the literature [2]. The C-terminal of the peptide was deprotected by catalytic hydrogenation in dichloromethane with 10 wt% palladium carbon and reacted with HFcB to afford BL16Fc. After that, the Boc group of BL16Fc (43 mg) was removed to afford HL16Fc. On the other hand, acetyl chloride was reacted to 4-thiobenzoic acid to yield 4-(S-thioacetyl)benzoic acid. The synthesized 4-(S-thioacetyl)benzoic acid (7.0 mg) was coupled to the prepared HL16Fc in chloroform in the presence of HATU (23 mg) and DIEA (16 μl) under N_2 atmosphere at 0 °C for 2 h and thereafter at room temperature for 24 h. After reaction, the solvent was evaporated, the crude product was purified by a Sephadex LH20 column (methanol as eluant), and washed with *n*-hexane to afford an orange powder (26 mg, 58 %). TLC: R_f (chloroform/methanol/acetic acid = 95/5/3 v/v/v) = 0.32, R_f (chloroform/methanol = 10/1 v/v) = 0.44. ^{1}H NMR (400 MHz, CD_3Cl): δ(ppm) 0.86 (48H, LeuCδH_3), 1.30-1.90 (75H, LeuCβH_2, LeuCγH, AibCH_3, AlaCβH_3), 2.43 (3H, s, CH_3CO), 3.31, 3.45, 3.62 (4H, brm, $NH(CH_2)_2NH$), 3.94 (9H, AlaCαH, LeuCαH), 4.14-5.11 (8H, ferrocenyl-H), 5.20 (2H, m, $OCH_2C_6H_5$), 6.97, 7.53-8.63 (23H, amide-H), 7.33 (5H, m, $OCH_2C_6H_5$). MS (FAB, matrix; nitrobenzylalcohol): m/z 2264 (calcd for $C_{113}H_{177}FeN_{19}NaO_{22}S$ $[(M+Na)^+]$ m/z 2264.2).

3.RESULTS AND DISCUSSION

3.1 Characterization of the Peptide Self-Assembled Monolayers

The peptide self-assembled monolayers (SAMs) were formed on gold, and the molecular orientation of the helical peptides was determined by FTIR reflection absorption spectroscopy. The tilt angles of the helices from the surface normal were 54° and 27° for the Fcl8FcL8SS SAM and the AcSL16Fc SAM, respectively. The relatively large tilt angle of the Fcl8FcL8SS SAM is probably due to the following two reasons: i) the C terminal octapeptide unit has a right-handed sense, but the N terminal is left-handed.

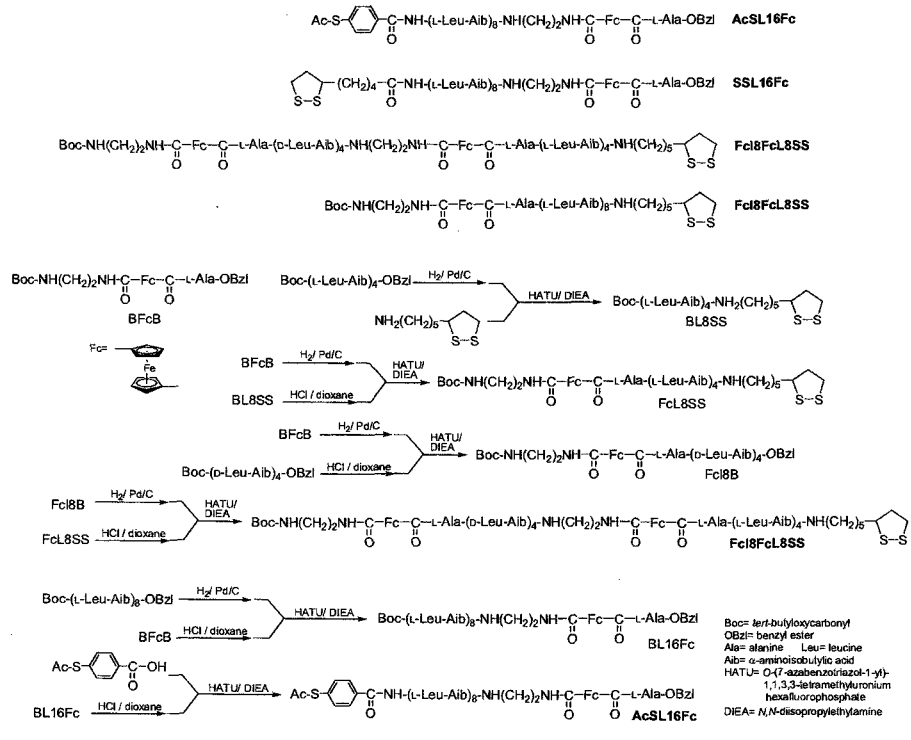

Fig. 1 Molecular structures of the helical peptides and the synthetic routes of Fcl8FcL8SS and AcSL16Fc.

This molecular design initially aimed at promotion of the phase separation between the upper helical layer and the lower helical layer with the intervening ferrocene layer. ii) The ferrocene unit in the middle of the molecule hinders formation of the intramolecular hydrogen bond. These two factors should prevent the peptide from taking a highly helical structure, resulting in a tilted molecular orientation with a loose packing.

3.2 Cyclic Voltammetry

Cyclic voltammetry was employed to investigate the oxidation of the Fc moiety on the SAM-modified substrates in a 1M $HClO_4$. At a scan rate of 100 mV/s a set of oxidative and reductive peaks were observed, suggesting that the electron transfer between the ferrocene unit and gold should be enhanced by the intervening ferrocene unit of Fcl8FcL8SS or the phenyl group at the linker moiety of AcSL16Fc, because no peak was observed with the SSL16Fc SAM at the scan rate due to the long-range electron transfer and the alkyl group at the linker moiety [2]. The surface coverages of the monolayers were estimated from the peak area of ferrocene oxidation to be $5.9 \pm 0.5 \times 10^{-11}$ mol cm^{-2} for the Fcl8FcL8SS SAM and $9.9 \pm 1.6 \times 10^{-11}$ mol cm^{-2} for the AcSL16Fc SAM. The coverage of the AcSL16Fc SAM is close to the theoretical value for a well-packed monolayer (10.9×10^{-11} mol cm^{-2}). On the other hand, the Fcl8FcL8SS SAM is considered to be loosely-packed. These are in a good agreement with the spectroscopic results by FTIR-RAS.

3.3 Electron Transfer Rate

The rate constants of the electron transfer reactions through the monolayer were examined by chronoamperometry at various overpotentials. In each case, single exponential decay of current with time was observed. Electron transfer among the ferrocene units is unlikely to occur because the distance among them is over 12 Å. The standard rate constants, which were obtained by extrapolation of the data to zero overpotential, are 9.2 s^{-1} for the Fcl8FcL8SS SAM and 29 s^{-1} for the AcSL16Fc SAM. The inelastic hopping mechanism should dominate over the superexchange mechanism in the long-range electron transfer reactions in the present SAMs.

Since the standard rate constant of the FcL16SS SAM was 0.68 s^{-1} [2], the electron transfer rate increased 14-fold larger by the introduction of the ferrocene unit in the middle of the helix (Fig. 2). The intervening ferrocene unit may relay the electron from the ferrocene unit at the N terminal to gold. However, a shorter distance between the ferrocene moiety and gold of the Fcl8FcL8SS SAM due to the large tilt angle may also explain the faster rate.

On the other hand, the replacement of the lipoic acid with the thiophenyl group at the linker moiety of the helical peptide resulted in 15-fold enhancement of the electron transfer rate (Fig. 2). The electron-transfer process from the molecule to gold should be the rate-determining step, and the π electrons of the thiophenyl group possibly promote the transfer.

Dependence of electron transfer rate constants on overpotentials for oxidation of the ferrocene moiety in the Fcl8FcL8SS SAM and the AcSL16Fc SAM was very weak. When the electron transfer occurred according to the superexchange mechanism, the rate dependence on the overpotential should be significant. This observation also supports the electron hopping mechanism in the present SAMs.

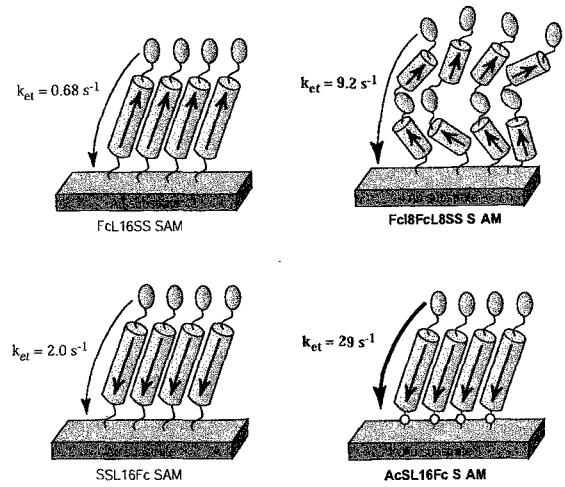

Fig. 2 The standard electron transfer rates of the helical peptide SAMs which were obtained by chronoamperometry.

4. Conclusion

The electron transfer along the helical peptide over 4 nm distance is found to be accelerated by the electron-relay group introduced in the middle of the molecule and by using the thiophenyl group at the linker moiety. The π-electron rich group is effective to promote electron transfer across electrode surface. The aromatic group also acts as an electron-relay group in the hopping mechanism. Helical peptides are useful for arrangement of the aromatic group at the junction, because the peptides are easily designed for a suitable scaffold. For example, we have designed a 3_{10} helix as a scaffold to arrange naphthyl groups linearly along the helix axis. With help of these naphthyl groups, the peptide has shown a good electron-mediating property [8]. We are now studying electronic properties of a single peptide molecule on gold.

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