Self-assembling chiral monolayers of helical peptides bound to gold *via* side-chain thioethers

Andrew E. Strong and Barry D. Moore*

Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, UK G1 1XL

A helical oligopeptide containing three methionine residues, positioned so their side-chains align along one side of the helix, forms self-assembled monolayers on gold as characterised by cyclic voltammetry and reflection–absorption IR spectroscopy.

Self-assembling monolayers (SAMs) of sulfur-containing molecules on gold are attractive systems for tailoring surface properties and the study of interfacial phenomena.[1] Our aim is to develop this technology so that ordered surfaces can be prepared featuring different functional groups positioned at relative predetermined positions on the nanometer scale. To this end we are investigating the formation and structure of self-assembling monolayers of helical oligopeptides. A few examples of self-assembled monolayers of peptides have recently appeared.[2,3] In all of these cases the peptides had only one strong attachment point to the gold surface, either through the side-chain of an N-terminal Cys residue[2] or *via* a thioalkyl carboxylic acid coupled to the N-terminal.[3] In order to obtain a defined alignment parallel to the surface we investigated binding a helical peptide to gold *via* multiple weaker interactions arising from methionine (Met) thioether side-chains. Peptides bound in this way provide maximum scope for organisation of other amino acids at the outer face to produce functionalised chiral surfaces.

Peptide **1**, Fcb-Ala-Aib-Ala-Met-Aib-Ala-Ala-Met-Ala-Aib-Met-Ala-Ala-NH_2, was designed so that in an α-helical conformation the thioether-containing side-chains of the three Met residues would align along the same side of the helix, able to bind to a gold surface. The remaining amino acids used, Ala and Aib (α-aminoisobutyric acid), are known strong helix formers in organic solvents[4] and have small methyl side-chains to minimise unfavourable steric interactions with the surface. The N-terminus was acylated with ferrocene butyric acid (Fcb) to provide a convenient electrochemical label. Peptide **2**, Fcp-Ala-Aib-Ala-Ala-Leu-Aib-Ala-Ala-Ala-Aib-Leu-Ala-NH_2, available from previous studies provided a convenient, non-sulfur-containing control of similar length, composition and secondary structure to **1**.

The peptides were prepared by conventional solid-phase peptide synthesis on a modified Rink-type resin using Fmoc chemistry and PyBOP® coupling protocols.† N-Acylation with the ferrocene carboxylic acids was carried out on the resin. Analysis of the cleaved precipitated products by reversed-phase HPLC and electrospray mass spectrometry‡ showed that in each case they were > 90% the desired peptide. Attempts to further purify peptide **1** invariably led instead to introduction of other impurities due to autooxidation of the methionine groups and hence the peptide was used freshly cleaved.

The CD spectra of peptide **1** in methanol and acetonitrile are shown in Fig. 1. The double minima at 208 and 222 nm are characteristic of a predominantly α-helical structure.[5] Analysis of the spectra[6] indicated that **1** was > 65% α-helical in methanol and 50% α-helical in acetonitrile. Other contributions can be assigned to 3_{10}-helix or random coil caused by fraying of the helix ends. Formation of the observed α-helix preorganises the three thioether side-chains to favour multipoint binding to a metal surface.

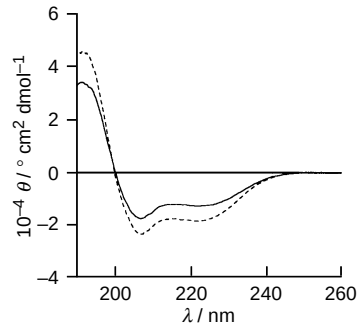

Fig. 1 CD spectra of oligopeptide **1** in MeCN (continuous line) and in MeOH (dashed line) at room temperature

Cyclic voltammetry was used as a convenient tool for monitoring the formation and relative stability of self-assembled monolayers of sulfur-containing peptide **1** compared to sulfur-free **2**. Ethanolic solutions of each peptide (*ca*. 1 mM) were contacted with a polished gold disk electrode for 20–26 h.§ Following rinsing with pure solvent, cyclic voltammograms were measured in ethanol (Fig. 2). Peaks characteristic of reversible electrochemistry of the covalently attached ferrocene were observed for both peptides. However the wave for peptide **2** was typically < 15% of peptide **1**.¶ Monolayers formed by peptide **1** were found to be remarkably stable to repetitive cycling. In fact, cycling appeared to condition the film so that the background current and peak to peak separation reduced while the Faradaic current remained constant [Fig. 2(*a*)]. In contrast the wave associated with the monolayer of peptide **2**

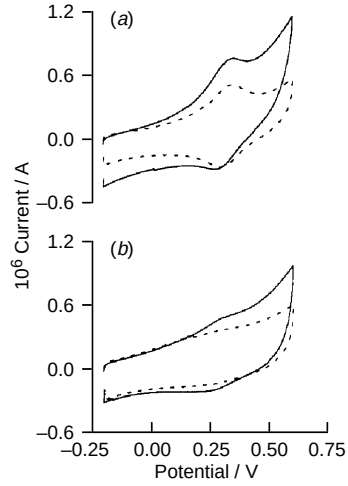

Fig. 2 (*a*) Cyclic voltammograms of films of peptide **1** adsorbed at a gold electrode, continuous line: recorded on immersion, dashed line: after sweeping for 30 min. (*b*) As above but for peptide **2**. The CVs were recorded at 0.5 V s^{-1} in 10 mM $NBu^n_4BF_4$ in EtOH *vs*. Ag/AgCl.

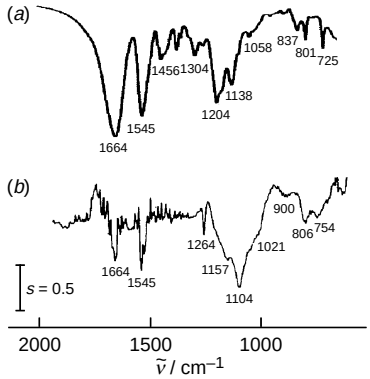

Fig. 3 IR spectra of oligopeptide **1**. (*a*) KBr spectrum and (*b*) reflection–absorption spectrum of the self-assembled film [scale bar applies to (*b*) only]

rapidly decreased during cycling under the same conditions [Fig. 2(*b*)]. The high stability of monolayers of **1** in ethanol, where hydrophobic interactions are absent, is consistent with the proposed mode of self-assembly *via* binding of the thioether side-chains to the gold surface.

The conformation and orientation of the adsorbed peptides were investigated by reflection–absorption IR spectroscopy (RAIRS).‖ The spectra of peptide **1** in a KBr pellet and self-assembled at gold** are shown in Fig. 3. The main indication of peptide secondary structure is the frequency of the amide I band (1600–1700 cm^{-1}).[7] Bands at 1620–1640 and above 1680 indicate β-structures while those at 1650–1658 are typical of α-helices. Interestingly the amide I band of peptide **1** appears at 1664 cm^{-1} close to that previously observed for 3_{10}-helices of Aib-containing peptides.[8]

The RAIRS technique also allows the orientation of the peptides at the surface to be inferred using the surface selection rule, because only vibrations with a component normal to the surface are enhanced.[9] The main component of the amide I band is stretching of the carbonyl groups and in a helical peptide these will be aligned along the helix axis. The main contributions to the amide II and III bands are in-plane bending of N–H and C=O, and C–C and C–N stretching.[10] For each of these vibrations the transition dipole moment subtends a range of angles to the helical axis. In the self-assembled peptide monolayers, binding *via* the methionine side-chains is expected to align the helical axis parallel to surface. This is expected to lead to a dramatic reduction in the intensity of the RAIRS amide I band relative to the amide II and III bands, as compared to the powder spectra. It can be seen in Fig. 3 that this is exactly what is found. The amide I band at 1664 cm^{-1} is significantly weaker in the reflectance spectra relative to the respective amide II and III bands at 1545 and 1264 cm^{-1}. RAIRS of peptide **2**, which lacks the thioether groups, showed weak bands consistent with a helical conformation at 1660, 1540 and 1300 cm^{-1} in the reflectance spectra. However their relative ratios were similar to those in the powder spectrum suggesting no particular orientation of the peptide at the surface.

We have shown peptide **1** adopts a helical secondary structure resulting in preorganisation of three surface binding thioether groups along one side of the helix. The peptide forms stable monolayers on gold with the helix aligned parallel to the surface. Future work will involve introduction of other amino acids into the sequence to produce functionalised chiral surfaces.

We thank EPSRC, Sharon Kelly and the BBSRC CD facility at Stirling University, Ras Raval and Elaine Cooper at University of Liverpool and Novabiochem.

Notes and References

* E-mail: b.d.moore@strath.ac.uk

† The oligopeptides were synthesised using a Novasyn Crystal solid-phase peptide synthesiser, Novasyn PR500 resin, Fmoc protected amino acids and PyBOP coupling chemistry. Each residue was double coupled using a threefold excess (for **1**) or a twofold excess (for **2**) of amino acid. Cleavage was with 10% TFA in CH_2Cl_2 for 2 × 45 min.

‡ Analytical HPLC was *via* reversed-phase HPLC on a C_8 column using an MeOH–H_2O gradient. ESMS data: peptide **1**, *m/z* 1417.6, found at *m/z* 1418.4 ($M + H^+$), 1400.7 ($M + H^+ - NH_3$), 740.9 ($M + Na^+ + K^+$), 732.6 ($M + 2Na^+$), 700.9 ($M + 2H^+ - NH_3$), 665.4 ($M + 2H^+ - Ala - NH_3$). Peptide **2**, *m/z* 1236.3, found at *m/z* 1274.8 ($M + K^+$), 1258.8 ($M + Na^+$), 1236.8 ($M + H^+$), 1219.8 ($M + H^+ - NH_3$), 638.1 ($M + K^+ + H^+$), 629.9 ($M + Na^+ + H^+$), 619.1 ($M + 2H^+$), 610.3 ($M + 2H^+ - NH_3$), 585.6 ($M + 2H^+ - Ala$).

§ The gold disk electrode (2 mm in diameter, purchased from Oxford Electrodes) was manually polished with alumina paste, rinsed with H_2O and immersed in 4 : 3 : 1 H_2O–conc. HCl–conc. HNO_3 for 60 s, rinsed with H_2O for 10 s and immersed in the peptide solution.

¶ A surface coverage of 17% was calculated modelling the helices as 27 × 12 Å rectangles and using a surface roughness of 2.

‖ RAIRS spectra were accumulated over 200 scans at 4 cm^{-1} resolution using a Unicam Galaxy Series FTIR 7000 spectrometer equipped with a Hg–Cd–Te detector and FT85 specular reflector from SpectraTech Inc. The sample compartment was purged with dry air.

** Peptide monolayers were assembled by evaporating 5 nm Cr and 50 nm Au onto a glass slide. The wafers were placed in 0.4 mmol dm^{-3} solutions of peptide **1** (in MeCN) for 16 h or peptide **2** (in CH_2Cl_2) for 4 h, rinsed with solvent and dried gently in a stream of He.

1 L. H. Dubois and R. G. Nuzzo, *Annu. Rev. Phys. Chem.*, 1992, **43**, 437 and references therein.
2 C. Duschl, M. Liley, G. Corradin and H. Vogel, *Biophys. J.*, 1994, **67**, 1229; M. Knichel, P. Heiduschka, W. Beck, G. Jung and W. Gopel, *Sensors Actuators B*, 1995, **28**, 85; C. Duschl, A. F. Sevinlandais and H. Vogel, *Biophys. J.*, 1996, **70**, 1985.
3 R. P. H. Kooyman, D. J. van den Heuvel, J. W. Drijfhout and G. W. Welling, *Thin Solid Films*, 1994, **244**, 913; J. K. Whitesell and H. K. Chang, *Science*, 1993, **261**, 73; S. Sakamoto, H. Aoyagi, N. Nakashima and H. Mihara, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2319; R. Naumann, A. Jonczyk, R. Kopp, J. van Esch, H. Ringsdorf, W. Knoll and P. Graber, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 2056.
4 I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747; C. Toniolo and E. Benedetti, *Trends Biochem. Sci.*, 1991, **16**, 350.
5 R. W. Woody, in *The Peptides*, ed. S. Udenfriend and J. Meienhofer, Academic Press, 1995, vol. 7, p. 15.
6 S. W. Provencher and J. Glöckner, *Biochemistry*, 1981, **20**, 33.
7 P. I. Haris and D. Chapman, *Biopolymers*, 1995, **37**, 251.
8 D. F. Kennedy, M. Crisma, C. Toniolo and D. Chapman, *Biochemistry*, 1991, **30**, 6451.
9 R. J. Greenler, *J. Chem. Phys.*, 1966, **44**, 310.
10 S. Krimm and J. Bandekar, *Adv. Protein Chem.*, 1986, **38**, 181.

Received in Bath, UK, 2nd December 1997; 7/08663F