A Surface Plasmon Resonance Study of the Binding of Antibody LDS47 to Self-assembled Monolayers of Cysteine Containing Peptides†

J. Chem. Research (S), 1999, 42–43†

Christine M. Bladon,* Johanna Kindermann, Nico A. J. M. Sommerdijk and John D. Wright

School of Physical Sciences, University of Kent, Canterbury CT2 7NH, UK

Surface plasmon resonance is used to monitor the kinetics of deposition and antibody binding of self-assembled monolayers of cysteine-containing peptides on gold surfaces.

Combinatorial chemistry is rapidly developing into a valuable method for the discovery of lead compounds in pharmaceutical and agrochemical research.[1,2] To date, however, the technique suffers from inefficiencies, particularly in the screening of immobilised libraries. Current assays often use labelled receptors to detect the recognition of active compounds. However the labelling itself may affect the interaction of the two components in the biospecific pair. Surface plasmon resonance (SPR)[3,4] has the potential to be developed into a screening method which requires no labelling and can also monitor receptor–ligand interactions in real time. It uses the angle-dependent reflectivity of a supported thin film of a metal to probe changes in the refractive index of any chemical layer attached to this metal film.[5] The principle of our approach, *i.e.* that the recognition between an immobilised peptide and an antibody can be detected by changes in the position and shape of SPR curves, is reported in this paper.

In this study the biospecific model pair consisted of the monoclonal antibody LDS47 and its decapeptide epitope sequence.[6] Two dodecapeptides, Ala-Pro-Gln-Ser-Ile-Thr-Glu-Leu-Cys-Ser-Gly-Gly **1** (the epitope decapeptide plus a spacer moiety of two additional glycine residues at the C-terminus) and the control sequence Ala-Ser-Pro-Ile-Thr-Gln-Glu-Leu-Cys-Ser-Gly-Gly **2**, were prepared using standard methods of Fmoc-*tert*-butyl solid phase synthesis.[7] In each of the two peptides the fourth residue from the C-terminus was cysteine in order to allow immobilisation to the SPR chip *via* sulfur–gold bonds.[8,9] Previous studies[10] had identified that the *N*-terminal alanine was the key residue for antibody recognition and this led to the choice of control sequence **2** which is a partially randomised form of the epitope peptide. The presence of the cysteine residue in the epitope sequence had also been found to be less important for antibody recognition and peptides featuring Cys(Acm) (Acm = acetomido), Cys(Me) or Ala at position 9 showed significant binding to LDS47. Consequently, although the interaction between the antibody and **1** immobilised to the gold layer of the sensor surface through the cysteine thiol was predicted to be weaker than with the epitope in free solution it was nevertheless expected to be detected using SPR.

The SPR experiments were carried out in real time, *i.e.* the sensor surface was continually in contact with a solution contained in a cell rather than being treated with the solution in question, dried, and then measurements taken. The sensor surface itself was a glass microscope slide (approximately 2 cm^2) which had been coated with a 40 nm thick layer of gold. A fresh slide was used for each SPR experiment. The slide was mounted on a 60° prism which

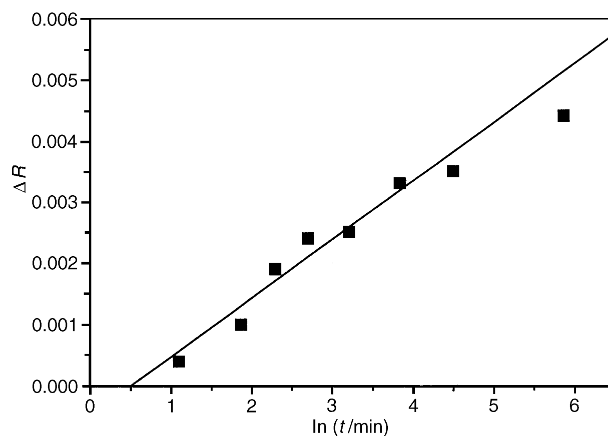

Fig. 1 Change in reflectivity (ΔR) due to deposition of a monolayer of **1** as a function of time measured at an angle of incidence of 5° with respect to the plane of the gold surface

was attached to a cell into which solutions could be injected. The peptide was deposited onto the gold surface by injecting an aqueous (phosphate buffer, pH 7.2) solution (5 ml) of **1** into the cell and formation of the monolayer was monitored by recording SPR curves at increasing time intervals. The initial change in reflectivity at a fixed angle of incidence was found to be linear with ln(time) (Fig. 1) indicating that the deposition of the peptide followed the Elovich equation which in turn implies that the rate of monolayer formation was dependent on the number of vacant sites.[11,12]

Excess **1** was removed from the cell and the slide, coated with the monolayer of immobilised peptide, was washed extensively with the buffer solution. A phosphate buffered solution of the antibody LDS47 (5 ml at 1 $\mu g\ ml^{-1}$) was injected into the cell and SPR curves recorded every few minutes for 30 min. A very slow change in the position of the resonance curve was observed. This was a rather disappointing observation as antibody binding was expected to lead to an increase in the thickness of the layer, thus resulting in a significant shift of the resonance curve. A possible explanation to account for the poor shift in resonance position was that the monolayer was too dense due to tight packing of the peptide molecules and that the bulky antibody was unable to recognise and therefore to bind to the epitope sequence. In a test of this hypothesis the epitope sequence was diluted in a two-dimensional manner by using solutions of different mixtures of **1** and cysteine for monolayer deposition. Using mixed monolayers deposited from solutions containing cysteine and peptide **1** in an approximate molar ratio of 4:1 the changes observed in the SPR curves upon addition of LDS47 were both larger and faster suggesting less hindered binding of the antibody to the isolated peptide molecules in the diluted layer (Fig. 2).

The selectivity of the recognition process for epitope **1** was investigated by comparison with the response of the

*To receive any correspondence (*e-mail:* C. M. Bladon@ukc.ac.uk).
†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1999, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

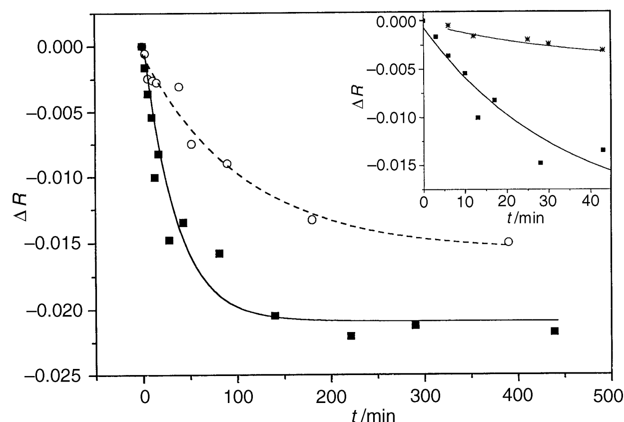

Fig. 2 Change in reflectivity (ΔR) due to binding of LDS47 to monolayers deposited from an aqueous solution of **1** (○) and from an aqueous solution of **1** containing 80% cysteine (■). Inset: Comparison of reflectivity changes due to binding of LDS47 to monolayers of **1** (■) and **2** (*) deposited from aqueous solutions containing 80% cysteine. Reflectivities were measured as a function of time at an angle of incidence of 7° with respect to the plane of the gold surface

system to control sequence **2**. Only a small response was observed upon addition of LDS47 to diluted monolayers of control peptide **2** (Fig. 2 inset).

In a final experiment the change in reflectivity at two fixed angles of incidence (either sides of the curve minimum) was monitored to give an indication of the real time binding of LDS47 to the diluted monolayer. A plot (Fig. 3) of reflectivity differences against ln(time) afforded a straight line for the initial period, again indicative of Elovich kinetics for binding by adsorption to vacant surface sites. However, after approximately 30 min the slope of the line changed suggesting a different mechanism for antibody deposition. This was interpreted as unspecific deposition of the antibody on the monolayer surface, which was supported by the fact that the latter process could be reversed by washing with buffer solution, whereas the initial binding proved to be irreversible.

This series of experiments has shown that SPR analysis of antibody–antigen interactions is both sensitive and selective. Moreover the process is quick; the entire procedure from forming the monolayer took approximately 2 h (20 min formation of monolayer, 60 min rinsing, and 30 min for antigen–antibody interaction). However, the immobilisation of large numbers of peptides through formation of Au–S bonds is clearly not feasible as a library screening process since each peptide would have to be made independently prior to attachment. The one common factor in both SPR and combinatorial chemistry is the solid support although the sensor surface of the former technique and the resins used routinely in the latter differ markedly in composition. We are currently constructing a solid support which is suitable not only for SPR analysis but is also compatible with solid phase peptide synthesis. Such a dual function material would offer real potential for the development of an effective SPR-based peptide library screening process.

Experimental

Peptide Synthesis.—Both peptides were assembled using standard Fmoc-*tert*-butyl solid phase chemistry[7] on a Novasyn TG resin bearing a 4-hydroxymethyl-3-methoxyphenoxyacetyl linker. Cysteine was protected as the Acm derivative during chain assembly. Crude peptides were analysed and purified using reverse phase HPLC and the pure compounds [**1**, Cys(Acm)] and [**2**, Cys(Acm)] were characterised by MALDI–TOF mass spectrometry (VG Tofspec). Compound **1** [Cys(Acm)]: found MH^+ 1233; $C_{50}H_{84}N_{14}O_{20}S$ requires MH^+ 1234. Compound **2** [Cys(Acm)]: found MH^+ 1233; calc. MH^+ *vide supra*. The Acm group was removed from both peptides with H_2S immediately prior to the SPR experiments.

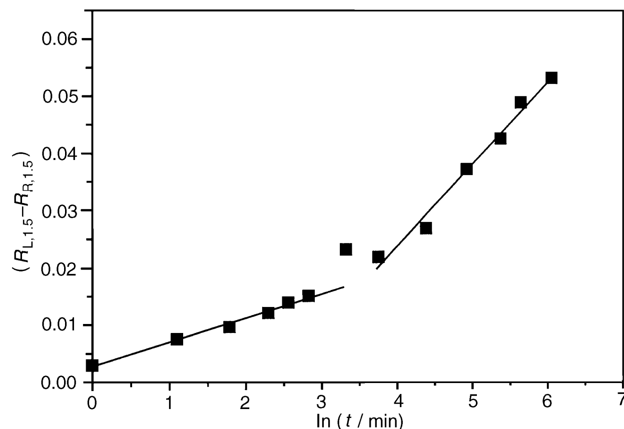

Fig. 3 Change of the difference in reflectivity at fixed angles of incidence (1.5° at either side from the minimum) due to binding of LDS47 to a monolayer deposited from an aqueous solution of **1** containing 80 mol% of cysteine. Reflectivities were measured at 8° ($R_{L,\,1.5}$) and 11° ($R_{R,\,1.5}$) with respect to the plane of the gold surface)

SPR Protocols.—SPR instrument comprised a Uniphase model 1125P 10mV helium neon laser, chopper, beam splitter, pinhole, rotating sample stage and two photodetectors. The HeNe laser beam ($\lambda = 632.8$ nm) passed first through the chopper to eliminate the effects of stray light and to produce a pulsed laser beam. After passing through the pinhole, the beam was diverted by a beam splitter. One of the laser beams was sent to the reference photodetector whilst the other passed into the 60° prism.

We are grateful to Kevin Howland and Judy Hardy of the Biosciences Department at UKC for their help with the mass spectral analyses.

Received, 17th August 1998; Accepted, 25th September 1998
Paper E/8/06441E

References

1 M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor and E. M. Gordon, *J. Med. Chem.*, 1994, **37**, 1233.
2 E. M. Gordon, R. W. Barrett, W. J. Dower, S. P. A. Fodor and M. A. Gallop, *J. Med. Chem.*, 1994, **37**, 1385.
3 A. Szabo, L. Stolz and R. Granzow, *Curr. Opin. Struct. Biol.*, 1995, **5**, 699.
4 P. Schuck, *Annu. Rev. Biophys. Biomol. Struct.*, 1997, **26**, 541.
5 B. Liedberg, C. Nylander and I. Lundström, *Sensors and Actuators*, 1983, **4**, 299.
6 T. Amin, A. Larkins, R. F. L. James and T. R. Hirst, *J. Biol. Chem.*, 1995, **270**, 20143.
7 E. Atherton and R. C. Sheppard, in *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford, 1989.
8 R. G. Nuzzo and D. L. Allara, *J. Am. Chem. Soc.*, 1983, **105**, 4481.
9 C. D. Bain, E. B. Troughton, Y.-T. Tao, J. Evall, G. M. Whitesides and R. G. Nuzzo, *J. Am. Chem. Soc.*, 1989, **111**, 321.
10 B. Giersing, MRes Thesis, University of Kent, 1996.
11 S. Y. Elovich and G. M. Zhabrova, *Zh. Fiz. Khim.*, 1939, **13**, 1761.
12 H. A. Taylor and N. Thon, *J. Am. Chem. Soc.*, 1952, **74**, 4169.