Engineered antibodies: readily adaptable molecular tools for basic and applied research

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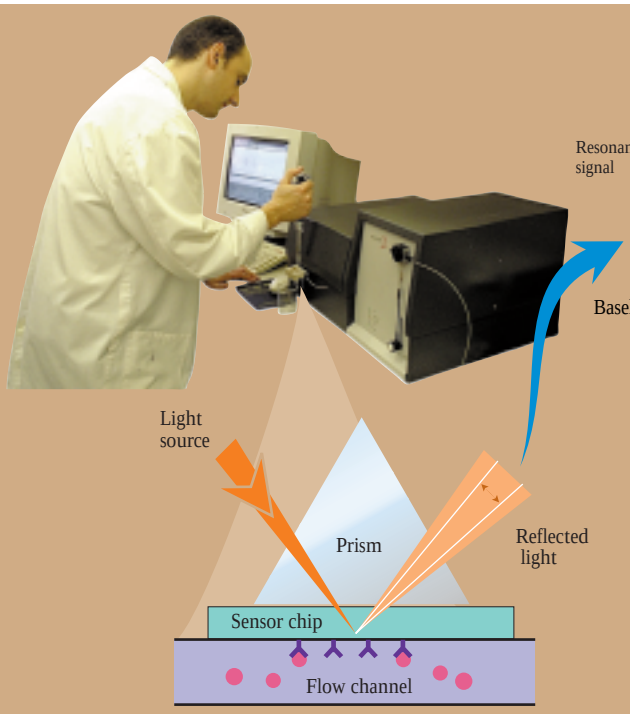
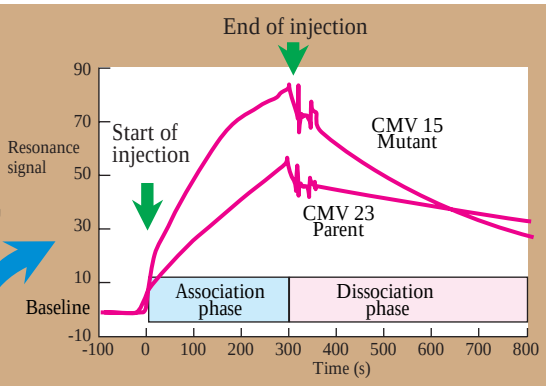
Introduction Antibodies are glycoproteins which have a modular structure with different domains having different functions. The variable domains that are responsible for specific binding to an antigen are in two chains, V_H in the heavy chain, and V_L in the light chain. We have cloned the genes encoding V_H and V_L and expressed them as a single polypeptide (scFv: single chain variable fragment) in *Escherichia coli* either on the surface of a bacteriophage (phage display), or as a soluble protein within the bacterial cells. Recent developments in antibody engineering have included the cloning of other domains from antibody molecules as well as other proteins, including enzymes, to express genetic scFv fusion proteins. These developments mean that it is possible to engineer different properties into the basic scFv molecule, such as multivalency to increase binding strength, bispecificity to broaden binding recognition, fusion to enzymes (or other reporter molecules) to facilitate detection of binding, and signal sequences to target expression to different sub-cellular compartments in plants.

Cloning and expression of antibody genes in heterologous hosts has opened up new areas for research, and new opportunities to devise novel diagnostics. For example, we have recently been successful in obtaining funding from the European Commission to explore the effects of expressing scFv in plants that bind to non-structural virus proteins, and from the DTI and industrial partners in the UK to develop novel diagnostics. The recent acquisition of a BIAcore X^{TM} instrument will allow accurate estimation of binding strength, and measure of the specificity of interactions of the novel antibodies (see box). This article





Sensorgram: mutant anti-cucumber mosaic virus (CMV) scFv
An anti-CMV recombinant scFv selected from a phage display library, CMV23, was compared with a mutant, CMV15, in which two amino acids in the variable domain were changed. CMV particles were immobilized on a dextran matrix by amine coupling, and scFv preparations were injected over the surface. CMV15 has a higher k_d than CMV23 and therefore was a poorer antibody.

The BIAcore X™ instrument (Biacore AB) measures interactions between molecules by surface plasmon resonance (SPR). The SPR response measures changes in mass at the detector surface, and thus the association or dissociation of the molecules. Test molecules can be immobilized to different surface matrices either directly by covalent bonds using amine, aldehyde or thiol groups, or indirectly through e.g. biotin/streptavidin linkages, or by binding to specific antibodies. The rates of association or dissociation are measured, and kinetic and equilibrium affinity constants are calculated from these.

The BIAcore X™ instrument, and principle of detection method.

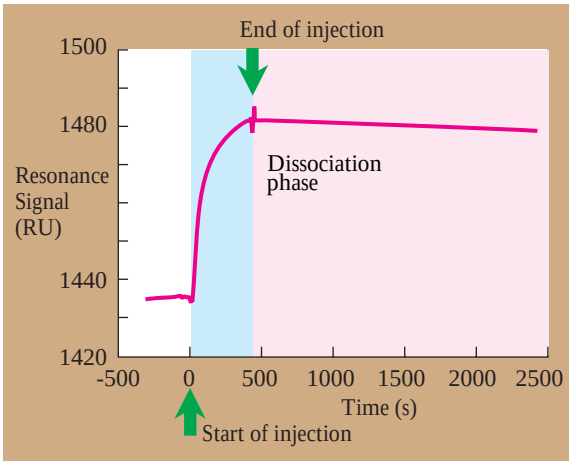
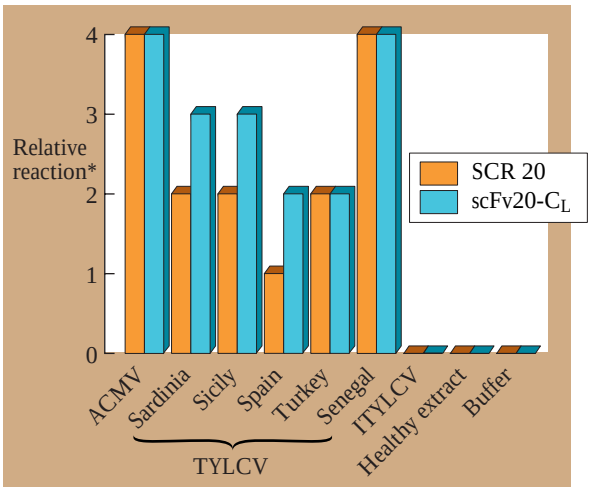


Figure 1 BIAcore X™ sensorgram of the interaction of MAb SCR20 with TYLCV.

describes some achievements of our current programme on novel diagnostics, and gives an indication of future research areas.

Novel diagnostics Whitefly-transmitted geminiviruses cause economically important yellow leaf curl diseases of tomato in many parts of the world. Different whitefly-transmitted geminiviruses have been shown to be serologically related and some monoclonal antibodies (MAbs) prepared against African cassava mosaic geminivirus were found to cross-react with viruses causing yellow leaf curl in tomato. In particular, MAb SCR 20 cross-reacts with tomato yellow leaf curl virus (TYLCV). When the binding of SCR 20 to a



Viruses were ACMV = African cassava mosaic; TYLCV = tomato yellow leaf curl isolates from five different countries; ITYLCV = Indian tomato yellow leaf curl virus. Tests were done by triple antibody sandwich ELISA in which virus was trapped from sap extracts by anti-ACMV antibodies and detected by either SCR 20 followed by anti-mouse-alkaline phosphatase conjugate or scFv-C_L followed by anti-CL-alkaline phosphatase conjugate.

*Absorbance values were ranked as follows: 4 = 1.21 -1.80; 3 = 0.61 -1.20; 2 = 0.31 - 0.60; 1 = 0.15 - 0.30; 0 = <2 x control.

Figure 2 Comparison of reactions in ELISA of geminiviruses with monoclonal antibody SCR 20 and recombinant scFv20-C_L.

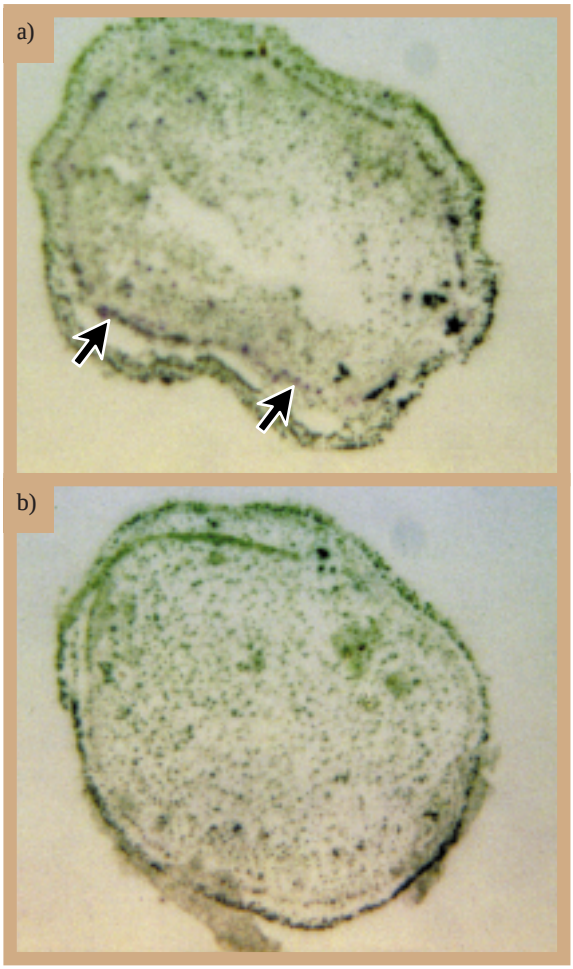


Figure 3 Detection of TYLCV in tomato stems by tissue print immunoblotting using scFv 20-C_L and a) infected b) healthy.

Sardinian isolate of TYLCV was investigated by surface plasmon resonance, we found that the dissociation of antibody molecules from the antigen was extremely slow, and the dissociation rate constant k_d was lower than 10^{-5} s^{-1} (Fig 1). The half-life of such an antibody-antigen complex is c. 19 hours.

The V genes of MAb SCR 20 were cloned and expressed as scFv fragments and as fusions to another part of the antibody molecule, the constant domain of

the light chain, C_L, forming scFv-C_L fusion proteins. The antibody fragments were readily expressed in *E. coli* and we found that they retained the same binding reactivity as the parent MAb (Fig 2). Tissue print immunoblotting tests using the scFv-C_L fusions showed that the virus was located in the vascular cells of tomato stem sections (Fig 3).

Phage display libraries In addition to cloning and expression of antibody genes from existing sources, it is also possible to obtain antibody genes from large phage display libraries of V genes. The phage antibodies are selected by binding to the target antigen immobilised on polystyrene tubes. The advantages of this approach are many but the most important of them is that we can be sure of a continuing supply of antibodies to virtually any antigen from a phage display library stored in the refrigerator. We obtained scFv fragments that bind potato leafroll virus (PLRV) from a phage display library after four rounds of selection on virus preparations immobilised on tubes. The scFv were sub-cloned such that the transformed *E. coli* secreted a scFv-alkaline phosphatase fusion protein. This antibody readily detected PLRV in extracts of infected potato plants with a sensitivity comparable to detection using conventional antibodies.

In addition, we have obtained scFv from phage libraries that bind to other plant viruses. These include blackcurrant reversion associated virus, a virus against which we were unable to obtain useful antisera by conventional methods. Also, scFv have been obtained to plant enzymes involved in lipid biosynthesis (enoyl acyl reductase) and starch synthesis (granule bound starch synthase; GBSS) from a synthetic phage display library.

Future prospects The properties of recombinant antibodies, such as fast selection of many different binders from a single stock, and the relative ease of their subsequent genetic manipulation, mean that they can be useful tools in several areas of basic research. An example is the incorporation of the genes into specially designed vectors for targeted plant expression. We are currently pursuing some aspects of scFv expression in plants, in particular the effects on virus replication and plant metabolism of expressing scFv that bind to virus encoded non-structural proteins, and to the enzyme GBSS. Another potentially productive research topic for future study may be to investigate the cellular location of novel genes. ScFv can be obtained that bind to short C-terminal peptide sequences translated from expressed sequence tags. These antibodies can be used in immunocytochemical studies to locate the putative protein, thus providing a clue to biological function of the gene.

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