Spectrum

Another look at the flu virus

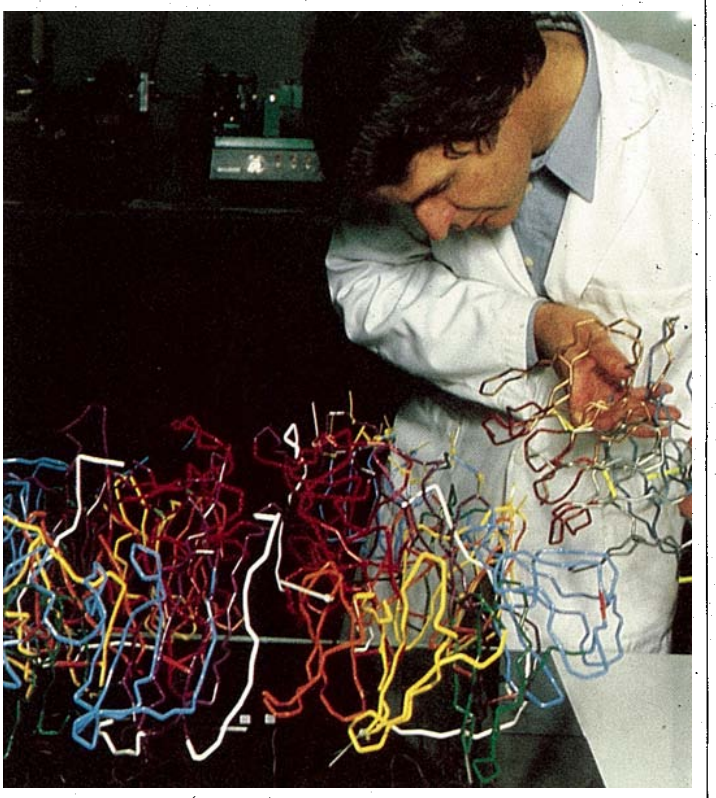
The influenza virus has experienced a most comprehensive dissection over the past 40 years. All of its genes have been sequenced, the protein products of those genes have been identified, and the frequent changes in the virus have been mapped. Because of their importance in the virus's evolution and interaction with man, two proteins embedded in the viral coat, haemagglutinin and neuraminidase, have attracted most interest.

Haemagglutinin is the protein responsible for binding the virus to the cell wall and facilitating its entry into the host cell; because of this crucial role, it receives the most attention from the body's immune system. *Ecos* 31 described research that has revealed its three-dimensional structure and given a detailed understanding of its changing nature.

Neuraminidase, an enzyme that splits a sugar off sugar–protein complexes, plays a supporting role to haemagglutinin — it facilitates the virus's exit from the infected cell. This influences the spread of the virus from cell to cell.

The body's immune system produces antibodies active against neuraminidase, but, since the virus does not require neuraminidase protein during the initial infection of a cell, these antibodies don't confer immunity from infection. Rather they act by restricting the spread of the virus and, as a consequence, reduce the severity of any attack of the flu.

Looking down on the box-like neuraminidase structure. The coloured arrows signify particular sequences of amino acids. The squares, triangles, and so on indicate where changes have occurred, and the stars indicate the active sites.



Constructing a model of neuraminidase.

The neuraminidase gene has been subject to the same evolutionary pressures as those experienced by the gene coding for the haemagglutinin protein. Antigenic drift, whereby regular mutations help the neuraminidase molecule sidestep the antibodies, occurs, as does antigenic shift — where it is suspected that recombination between different flu viruses (such as those infecting ducks and man) introduces a totally new neuraminidase molecule into the human flu virus.

Scientists have identified the changes that have occurred in the neuraminidase gene during antigenic drift and shift. This was an international effort in which CSIRO scientists Dr Colin Ward, Dr Ahmed Azad, and Dr Tom Ellerman, from the Division of Protein Chemistry, participated.

Now, in an important advance, colleagues in the Division, Dr Peter Colman and Dr Jose Varghese, and Dr Graeme Laver of the John Curtin School of Medical Research in Canberra, have used this information and the technique of X-ray crystallography to piece together a three-dimensional picture of the neuraminidase molecule. This shows where the major changes occur and the positions of the active sites — or those parts of the molecule that are responsible for its activity.

Like the haemagglutinin molecule, neuraminidase is embedded in the membrane that surrounds the virus. This membrane is derived from the cell that the virus has invaded. Four sub-units combine to form the complete neuraminidase molecule, which extends out of the membrane on a slender stalk before expanding into a box-shaped head; small areas at the four corners of the box each constitute an active site.

This site does not change with antigenic drift; any change would interfere with its functioning and threaten the virus's survival. According to the scientists, another reason why it may not be subject to change is that antibodies cannot reach it — each active site is in a narrow cavity, and antibodies are too large to reach into such a cleft.

Because of the protected nature of the active site it is unlikely that scientists can construct synthetic vaccines directed against it. However, they may be able to develop anti-flu drugs whose own active site is a small projection that binds to neuraminidase's active site — but, of course, this would involve a long and expensive research effort.

Most importantly, the unravelling of the structure and function of both haemagglutinin and neuraminidase has given scientists the most comprehensive description so far of the surface structure of any virus. The unique vantage point that this provides will be invaluable in modelling the dynamic relation between viruses and their hosts. In particular, it should make possible a better understanding of how antibodies work.

At the moment, interest in using synthetic antibodies derived from genetically engineered bacteria is widespread. However, a general ignorance obscures the rules that govern antibody activity.

To discover some of these rules, Dr Colman has obtained purified crystals of antibodies active against neuraminidase prepared by Dr Laver and two scientists in the United States, Dr Robert Webster of the St Jude's Children's Hospital in Memphis, Tennessee, and Dr Gillian Air, an Australian working at the University of Alabama. Dr Colman is now subjecting these antibodies to X-ray diffraction studies. The results could provide the most complete picture yet of how antibodies interact with their target protein.

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Structure of the influenza virus glycoprotein antigen neuraminidase at 2·9A resolution. J.N. Varghese, W.G. Laver, and P.M. Colman. *Nature*, 1983, **303**, 35–40.

Structure of the catalytic and antigenic sites in influenza virus neuraminidases. P.M. Colman, J.N. Varghese, and W.G. Laver. *Nature*, 1983, **303**, 41–4.