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VIRAL ANTIGEN-ANTIBODY COMPLEX

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# nature

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Crystals of a complex of antibody Fab fragment and influenza virus neuraminidase. Photograph by Julie Macklin and Stewart Butterworth in backlighting to illuminate different crystal facets in different colours. See p.358 and News and Views p.334.

## THIS WEEK

### ■ Sombre view of AIDS ... page 343

A worst-case study shows a surprisingly high fraction of those infected with the AIDS virus may go on to develop the full disease.

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Meanwhile, a deal between the French and US sides in the AIDS patents dispute will give a boost to AIDS research by funding a new research centre.

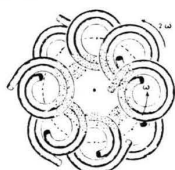
### ■ Population stability ... pages 328 and 388

The belief that greater complexity leads to greater ecological stability is challenged by the observation that spatial heterogeneity destabilizes the interactions between aphids and predatory ladybirds.

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Electroreceptors in the bill of the duck-billed platypus can pick up muscular electrical activity from the tail flick of the freshwater shrimp on which it feeds.

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13. Stoeckel, K., Schwab, M. & Thoenen, H. *Brain Res.* **89**, 1-14 (1974).
14. Angeletti, R. H. & Bradshaw, R. A. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2417-2420 (1971).
15. Scott, J. *et al. Nature* **302**, 538-540 (1983).
16. Ullrich, A., Gray, A., Berman, C. & Dull, T. J. *Nature* **303**, 821-825 (1983).
17. Darling, T. L. J. *et al. Cold Spring Harb. Symp. quant. Biol.* **48**, 427-434 (1983).
18. Menescini-Chen, M. G., Chen, J. S. & Levi-Montalcini, R. *Archs ital. Biol.* **116**, 53-84 (1978).
19. Letourneau, P. C. *Devl Biol.* **66**, 183-196 (1978).
20. Gundersen, R. W. & Barrett, J. N. *Science* **206**, 1079-1080 (1979).
21. Levi-Montalcini, R. *Prog. Brain Res.* **45**, 235-258 (1976).
22. Rencnan, W. E. & Munger, B. L. *J. comp. Neurol.* **246**, 129-145 (1986).
23. Iggo, A. *Br. med. Bull.* **33**, 97-102 (1977).
24. Iggo, A. & Andres, K. H. A. *Rev. Neurosci.* **5**, 1-31 (1982).
25. Davies, A. M. & Lumsden, A. G. S. *J. comp. Neurol.* **223**, 124-137 (1984).
26. Davies, A. M. & Lumsden, A. G. S. *J. comp. Neurol.* **253**, 13-24 (1986).
27. Korsching, S. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3513-3516 (1983).
28. Bandtlow, C., Heumann, R., Schwab, M. & Thoenen, H. *EMBO J.* (in the press).
29. Rush, R. A. *Nature* **312**, 364-367 (1984).
30. Finn, P. J., Ferguson, I. A., Renton, F. J. & Rush, R. A. *J. Neurocytol.* **15**, 169-176 (1986).
31. Lumsden, A. G. S. & Davies, A. M. *Nature* **306**, 786-788 (1983).
32. Lumsden, A. G. S. & Davies, A. M. *Nature* **323**, 538-539 (1986).
33. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).

## Three-dimensional structure of a complex of antibody with influenza virus neuraminidase

P. M. Colman, W. G. Laver\*, J. N. Varghese, A. T. Baker, P. A. Tulloch, G. M. Air† & R. G. Webster‡

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*The structure of a complex between influenza virus neuraminidase and an antibody displays features inconsistent with the inflexible 'lock and key' model of antigen-antibody binding. The structure of the antigen changes on binding, and that of the antibody may also change; the interaction therefore has some of the character of a handshake.*

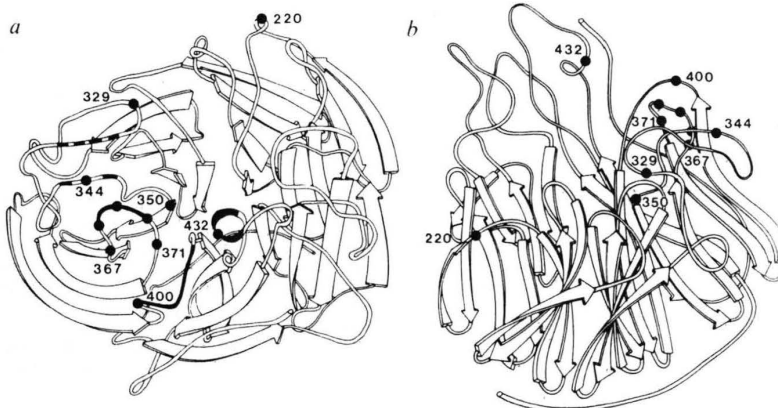
WE report here the first analysis by X-ray diffraction of the three-dimensional structure of a viral antigen complexed with an antibody Fab fragment. The antigen is the influenza virus enzyme neuraminidase (reviewed in ref. 1).

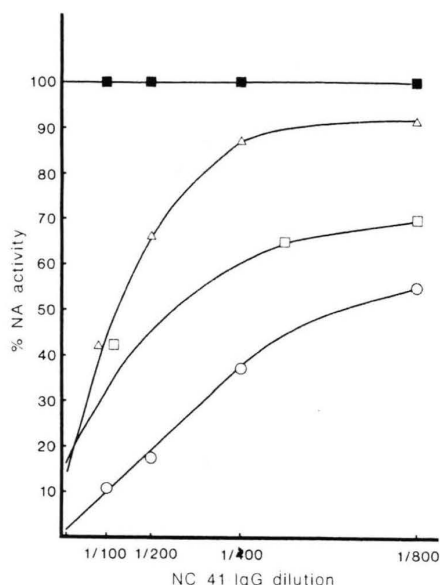
Sufficient data on the chemical<sup>2</sup> and spatial<sup>3-6</sup> structure of antibodies emerged in the 1970s to provide a basis for understanding how variation in antibody structure occurs, but the question of how different antibodies accommodate different macromolecular epitopes remains unresolved. The antigen binding fragments (Fab) of immunoglobulins consist of a light chain (L) and the N-terminal half of the heavy (H) chain. On each chain there are two globular domains of ~100 amino acids, the N-terminal domain in each chain being variable (V<sub>L</sub> and V<sub>H</sub>) and the C-terminal domain conserved (C<sub>L</sub> and C<sub>H</sub>1) in their amino-acid sequences. V<sub>L</sub> and V<sub>H</sub> domains are associated with each other, forming a variable module and C<sub>L</sub> and C<sub>H</sub>1 form the constant module. Three complementarity-determining regions (CDR1, 2 and 3) from each of the V<sub>L</sub> and V<sub>H</sub> domains are clustered at the extremity of the Fab arms, and

they determine the binding specificity of an immunoglobulin molecule.

The three-dimensional structures of five free Fab fragments<sup>7-11</sup> and one in complex with lysozyme<sup>12</sup> have been reported. Four of the structures, New<sup>7</sup>, Kol<sup>8</sup>, McPC603<sup>9</sup> and J539<sup>10</sup>, are well refined and form the basis of the following generalizations which also appear valid for the other two, S10/1 (ref. 11) and D1.3 (ref. 12). The pairing of V<sub>L</sub> and V<sub>H</sub> domains is determined by amino acids from both the framework region and the CDRs. Most of the surface area buried in the interface derives from conserved residues and this observation explains the largely conserved geometry of V<sub>L</sub>-V<sub>H</sub> pairing<sup>13</sup>. Small differences in pairing presumably result from interactions among the CDR amino acids<sup>14</sup>. Analysis of the V<sub>L</sub>-V<sub>H</sub> contact surface shows it to be unlike other interfaces between β-sheets<sup>15</sup>. Amino acids from the outermost strands of the two sheets fold into the interface where they contribute the bulk of the buried surface. C<sub>L</sub>-C<sub>H</sub>1 dimers are similarly conserved in their association pattern<sup>16</sup>. More variable is the so-called elbow angle<sup>17,18</sup>

**Fig. 1** Schematic diagrams of chain fold in N2 and N9 neuraminidase viewed down the 4-fold axis (a) and perpendicular to this axis (b). The symmetry axis is bottom right in a and standing vertical at the left rear in b. The view of the neuraminidase in b is similar to that shown in Fig. 5. Tagged residues and adjacent chain segments are referred to in the text. N2 numbering is used. In a, the side chains of amino acids 368-370 point towards the viewer, whilst that of Arg 371 points away and into the catalytic site located above and to the right of C<sub>α</sub> 371. Mutations at positions 367, 369, 370, 400, and 432, as detailed in Fig. 2, abolish the binding of NC41 antibody to neuraminidase, whereas mutations at 368 and 329 reduce that binding. A mutation at 220, which falls outside the NC41 epitope, has no effect on NC41 binding to neuraminidase (see Fig. 2). In a, solid chain segments show regions in contact with the NC41 antibody; contact assignments in the broken solid segments are tentative.





**Fig. 2** Neuraminidase inhibition (NI) curves of monoclonal antibody NC41 acting on N9 neuraminidase (NA) and a number of variants selected with different monoclonal antibodies to N9 neuraminidase. Assays used fetuin as a substrate, as described<sup>58</sup>. The amino-acid sequence changes of the variants are: ■, 434 (432 in N2 numbering) K → N; 400 (400) N → K; 371 (370) S → L; 370 (369) A → D; 368 (367) S → N; □, 369 (368) I → R; △, 331 (329) N → D (designated as OX2 in text); ○, 222 (220) R → Q (this curve is identical to wild-type). The amino-acid sequence changes were determined by sequencing cDNA of the viral RNA strand encoding the neuraminidase as described<sup>59</sup>. The changes are shown on the 3-D structure of the monomer in Fig. 1.

describing the angle between the local axes of symmetry in the V and C modules.

The identification of antigenic regions on protein molecules<sup>19</sup> has been attempted by a variety of methods<sup>20-23</sup> but the structural basis of antigenicity remains unclear. Current models of antigen-antibody association are based on classical ideas of lock and key complementarity. But it has been suggested<sup>24</sup> that the antigenic regions of proteins are located in the more flexible chain segments, which may adopt a configuration that allows antibody binding. It is not known whether binding to antigen has any effect on the structure of an antibody<sup>25</sup>, nor whether, if such an effect exists, it has biological significance. For the Fab-lysozyme complex<sup>12</sup>, the parts of the lysozyme that make contact with the antibody are not the more flexible regions of free lysozyme. No deformation of the antigen nor any structural change in the Fab was observed<sup>12</sup>.

The antigen in this study is the neuraminidase of influenza virus. It is a tetramer of subunits with relative molecular mass 60,000 ( $M_r$  60 K) with circular symmetry, and is attached by a stalk to the viral membrane. Neuraminidase 'heads' can be liberated from the virus by proteolysis<sup>26</sup>. There is antigenic variation in the neuraminidase between different strains of virus. Antibodies against neuraminidase do not neutralise virus infectivity, but do modify the disease in favour of the host<sup>27</sup>. The three-dimensional structures of neuraminidase heads of subtypes<sup>28</sup> N2 (refs 29, 30) and N9 (A.T.B., J.N.V., W.G.L., G.M.A. and P.M.C., manuscript in preparation) are known and a schematic of the chain fold is shown in Fig. 1. Neuraminidase of subtype N9 from an avian influenza virus, G70c<sup>31</sup>, has been used in this study. Its structure is very similar to the N2 enzyme as expected from the sequence homology of 50% (ref. 32).

We previously reported electron and low resolution X-ray diffraction studies of Fab-neuraminidase complexes<sup>33</sup> and more

recently, the crystallization of another complex (with NC41 Fab) which diffracts X-rays to beyond 3 Å resolution<sup>34</sup>. The NC41 antibody can suppress the yield of virus from infected cells and therefore allows the selection of antigenic variants. Neuraminidase-inhibition curves (Fig. 2) show that some variants with single sequence changes are not inhibited at all by NC41 antibody whereas others are partially inhibited. The location of these sequence changes on the three-dimensional structure of neuraminidase is shown in Fig. 1.

### Protomer structure

Electron microscopy of negatively stained protomers of complex show tetrameric neuraminidase heads with four Fab fragments attached (data not shown). Their image appearance is very similar to that of protomers of N9 neuraminidase complexed with the Fab fragments of antibodies 32/3 and NC35 (ref. 33) and may be described as a square box ( $100 \times 100 \times 60$  Å) with four antennae (Fab molecules) attached to one surface on the outer corners. The overall shape of the protomer from the X-ray diffraction study (Fig. 3) is consistent with the electron microscope images. The Fab arms subtend an angle of 45° to the plane of the tetrameric neuraminidase head, slightly foreshortened in the electron microscope image<sup>33</sup>.

Surface loops of the neuraminidase in contact with the CDRs are 368-370, 400-403, 430-434 and parts of 325-350. Of these, the conformation of the 325-350 loop is still tentative. It was the most difficult region of the original N2 neuraminidase structure<sup>29</sup> to interpret and remains ambiguous in the N9 neuraminidase structure (A.T.B. *et al.*, manuscript in preparation). Such instances of ill-defined structure are typical of surface loops of polypeptides subject to thermal motion or statistical disorder.

Early indications on the distribution of temperature factors in the uncomplexed N2 neuraminidase, based on crystallographic refinement of that structure at 2.2 Å resolution and an  $R$ -factor of 0.293, show above-average thermal parameters in chain segments 109-111, 141-142, 221-222, 243-247, 305-309, 316-319, 327-331, 338-343, 365-369, 399-402, 429-437 and 461-469 (J.N.V. and P.M.C., unpublished data). Elevated  $B$  values around residue 330 may correlate with inaccurate modelling as discussed above. All the segments listed here are surface loops. Note that only one of the hotter loops is underneath the neuraminidase head. We emphasize that the temperature factor analysis is preliminary, and indeed relates to N2, not N9, neuraminidase.

The monoclonal variant of N9 neuraminidase known as OX2 (Asn 329 → Asp 329, N2 numbering) crystallizes isomorphously with wild-type N9-NC41 Fab complex<sup>34</sup>. NC41 inhibits the OX2 neuraminidase variant less than it does wild-type (Fig. 2). Refinement of the N9-NC41 Fab structure using data collected from the OX2-NC41 Fab complex yields an  $R$ -factor of 0.36 for a model with tight geometry. This result is comparable with the current  $R$ -factor against the native dataset and confirms our earlier conclusion<sup>34</sup> that the binding of NC41 Fab to wild-type and OX2 N9 neuraminidases is isosteric. Either some bonding 'glue' is absent or repulsive forces have been introduced by the mutation (Fig. 2), but there is no detectable rearrangement of the antibody on the antigen at this stage. The precise location of residue 329 (N2 numbering) in the N2 and N9 structures is unclear. Refinement of the OX2-NC41 Fab structure should eventually reveal the structural basis for the reduced binding of NC41 to OX2 compared with wild-type N9.

The third CDRs on both heavy and light chains are not yet completely modelled, and the conformation of the 325-350 region on the antigen remains uncertain, so we cannot identify the amino acids that interact in the interface. All the CDRs, with the possible exception of light-chain CDR1 appear to make contact with the epitope. A preliminary assessment is that there are no more contacting residues than the 16 or 17 observed in the Fab-lysozyme structure<sup>12</sup>.

## Quaternary structure of the Fab

The amino-acid sequence of the Fab is known (G.M.A., unpublished data). The assignment of heavy and light chains in the Fab structure is consistent with side-chain densities at homologous positions on the two chains, such as heavy-chain (H) Trp 47 and light-chain (L) Leu 46, H Val 37 and L Tyr 36, and, H Trp 103 and L Phe 98. Heavy-atom binding sites also show an asymmetry which is consistent with these observations.

The quaternary structures of known Fab fragments were compared as described in Table 1. The lower left triangle of entries indicates that  $C_L$ - $C_{H1}$  pairing is a function of immunoglobulin chain subtype since  $\gamma_1$ : $\lambda$  chains (as in Kol and New) show a consistent mode of association as do  $\alpha$  or  $\gamma_{2a}$ : $\kappa$  chains (as in M603 and NC41). Small but significant alterations in V-domain pairing are observed between Kol, New and M603 (Table 1, upper right). This variation in  $V_L$ - $V_H$  association has been attributed to the fact that the CDRs contribute partially to the dimer interface between the two domains in the V-module<sup>14</sup>, although in the case of Kol we suggest that intimate crystal contacts involving the CDRs may contribute.

Comparison of these three Fabs with NC41 complexed with neuraminidase shows, in each case, a significantly larger difference in  $V_L$ - $V_H$  association. Compared with Kol, the distance between H CDR2 and L CDR3 is larger and the relative positions of the CDRs around the antigen-binding surface are altered by up to 4 Å. A movement of this magnitude is of the order of the distance between adjacent  $\alpha$ -carbon atoms on a polypeptide and may be considered as dramatic as a sequence insertion or deletion in a structurally critical part of the molecule. As well as the bulk movement of domains described here, the CDR loops may be flexible and able to adapt further to an epitope.

Building an atomic model into the penultimate electron density map (that in which no phase information from the Fab structure had been included) required the rotation of H Trp 47 180° around  $C_\alpha$ - $C_\beta$  compared with its position in Kol. M603 and New are the same as Kol at this point. The positions of the loops at residue 40 on both heavy and light chains also required remodelling. Therefore the reorganization of the interface through sliding of domains has consequences for the domain structure of the  $V_L$  and  $V_H$ .

The sequence of the NC41- $V_L$  and  $V_H$  domains shows that those framework amino acids on both the light chain (Tyr 36, Gln 38, Pro 44, Tyr 87, Phe 98) and the heavy chain (Val 37, Gln 39, Leu 45, Trp 47, Phe 91, Ala 93, Trp 103) that are buried in the interface<sup>15</sup> are conserved as expected. There is nothing in the primary sequences of the CDRs of NC41 to suggest a cause of the unusual domain association. An independent analysis of the NC41 sequence confirms this view (C. Chothia, personal communication).

Two other Fab structures are known to fit the normal pattern of  $V_L$ - $V_H$  association, the free antineuraminidase monoclonal antibody S10/1 (ref. 11) and the complexed anti-lysozyme monoclonal antibody D1.3 (ref. 12). Among light-chain dimers the picture is less clear. Although several examples of  $V_L$ - $V_H$ -like association have been observed<sup>3,35-37</sup>, there are two clear exceptions<sup>38-39</sup>, one of which is said to be caused by the CDR residue L 91, or, in our view, perhaps by a His at residue 38.

If changes in the amino-acid sequence of the CDRs (which are only a small part of the interface) can modulate the pairing pattern, binding of the six CDRs to a macromolecular surface might similarly perturb the  $V_L$ - $V_H$  interface. Without knowing the three-dimensional structure of the free NC41 Fab we cannot definitely conclude that the antibody structure has changed on binding antigen, but we believe that this explanation is more plausible than the alternative, which requires that special sequences in the CDRs alone have determined the pairing pattern.

Other data support conformational changes in antibodies when antigen or hapten binds<sup>40-44</sup>. Changes in circular dichro-

Table 1 Quaternary structure comparisons of Fabs

	NEW	M603	KOL	NC41
NEW		4.2 (0.7)	6.5 (0.4)	12.2 (1.0)
M603	12.7 (2.3)		5.0 (0.3)	8.8 (1.3)
KOL	2.5 (0.0)	12.4 (2.4)		12.1 (0.7)
NC41	11.5 (0.4)	2.1 (0.7)	10.8 (2.1)	

Comparison of four different Fab quaternary structures. Upper right triangle:  $V_L$  domain of the row Fab was mapped into the  $V_L$  domain of the column Fab using the  $C_\alpha$  coordinates of residues 33-39, 43-47, 84-90, 98-104 (Kabat<sup>2</sup> numbering). These amino acids were chosen because they define the interface between  $V_L$  and  $V_H$  domains<sup>15</sup>. The calculated transformation was applied to the  $V_H$  domain of the row Fab and the additional rotation in degrees (and translation in Å) to optimize overlap of the two  $V_H$  domains in question was calculated and is given above.  $C_\alpha$  coordinates used for overlap of  $V_H$  domains were 34-40, 44-48, 88-94 and 103-109. Lower left triangle:  $C_L$  domain of the column Fab was mapped into the  $C_L$  domain of the row Fab using the  $C_\alpha$  coordinates of residues 118-124, 131-137, 160-162 and 174-178 (Kabat<sup>2</sup> numbering). These amino acids were chosen because they define the interface between  $C_L$  and  $C_{H1}$  domains<sup>16</sup>. The calculated transformation was applied to the  $C_{H1}$  domain of the column Fab and the additional rotation in degrees (and translation in Å) to optimize overlap of the two  $C_{H1}$  domains are shown.  $C_\alpha$  coordinates used for overlap of  $C_{H1}$  domains were 121-125, 139-145, 172-179 and 186-190. Amino acids from the interface were used because if this set is expanded to include other parts of the domain structure, the correlation between small rotation angles and similar chain classes that is observed for the C module is less good. The largest r.m.s. distance between domain structure alignments is 0.7 Å. A similar comparison was done for the V modules of the M603 (antiphosphocholine) and uncomplexed S10/1 (antineuraminidase) Fab fragments. No measurable difference in the way the  $V_L$  and  $V_H$  domains associate in those antibodies was detected. A comparison elsewhere of Kol and New V modules<sup>8</sup> yields a value of 9°. The value reported here (6.5°) is smaller because it is uninfluenced by structural differences outside of the interface zone.

ism<sup>40</sup> or circular polarization of fluorescence<sup>42</sup> indicate an altered environment for aromatic residues after antigen binds to antibody, and kinetic data support a bi-molecular process with distinct conformational states for bound and free Fab<sup>41,43,44</sup>. Both of these aspects are embodied in the sliding  $V_L$ - $V_H$  model presented here.

Direct structural evidence has been presented for hapten-induced structural changes in a Bence-Jones protein<sup>45,46</sup>. In that case hydrophobic ligands can apparently penetrate the  $V_L$ - $V_L$  interface and signal their presence to the C-module. Strain introduced in the structure of the C-module can be relaxed by reducing and re-oxidizing the disulphide bond between the C-terminal regions of the two light chains<sup>46</sup>. Table 1 indicates that in the neuraminidase-NC41 complex no significant changes in the quaternary structure of the C-module have occurred.

Analysis of the pseudo-symmetry axes of the V and C modules shows that the angle between these axes, the elbow angle, is ~150°, which is intermediate in the observed range of 130-180° for other Fab structures. The sense of the bending is the same as in all other Fab fragments, with  $V_H$  and  $C_{H1}$  domains closer together than the  $V_L$  and  $C_L$  domains.

## Conformational changes in the antigen

The neuraminidase upper surface loop involving residues 367-371 was remodelled to better fit the density as observed in the complex. Adjacent parts of the loop which were also omitted from the phasing process (residues 364-366 and 372-373) fit the density as if they are rigidly attached to the N9 core. The  $C_\alpha$  atoms of residues 368-371 have been moved by 1 Å or more from their position in N9 neuraminidase (Fig. 4). Least-squares refinement of the structure to  $R=0.35$ , gave a mean shift of 0.4 Å in all  $C_\alpha$  atoms with a standard deviation of 0.2 Å. The current position of the 370 loop (Fig. 4) shows displacements of more than 1.2 Å for  $C_\alpha$  atoms 369-371 compared with their

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