Do Antibodies Prefer Moving Targets?

Antibody recognition of their targets may be enhanced because they preferentially bind to the more mobile segments of protein antigens

Although a great deal is now known about antibody synthesis and assembly the final stage of antibody action remains shrouded in mystery. The question of exactly what an antibody "sees" when it recognizes its antigen has not been fully answered. Without the answer a complete understanding of how the immune system distinguishes between foreign and indigenous molecules is not possible.

Recently, two independent groups, using different approaches, have come to the same, somewhat surprising, conclusion about antibody recognition of protein antigens. Their results indicate that antibodies preferentially bind to the more mobile segments of protein molecules. "Antigen-antibody union is more like two clouds coming together than two rocks," as Richard Lerner of the Research Institute of Scripps Clinic puts it. This contrasts with the theory that antibodies recognize rigid shapes on antigens, just as a key fits a lock.

The new results may help to explain how antibodies can recognize a virtually unlimited number of antigens. The immune system is capable of generating a very diverse population of antibodies that can fit with and bind to a wide range of antigens. Nevertheless, a preference for the more mobile portions of their targets would provide an additional layer of insurance that antibodies could tackle every possible antigen. The fit would not have to be absolutely perfect to begin with if the antibody bound to a flexible portion of the antigen. "You can't expect an antibody to fit every situation. The protein accommodates to the antibody," explains Aaron Klug of the MRC Laboratory of Molecular Biology in Cambridge, England.

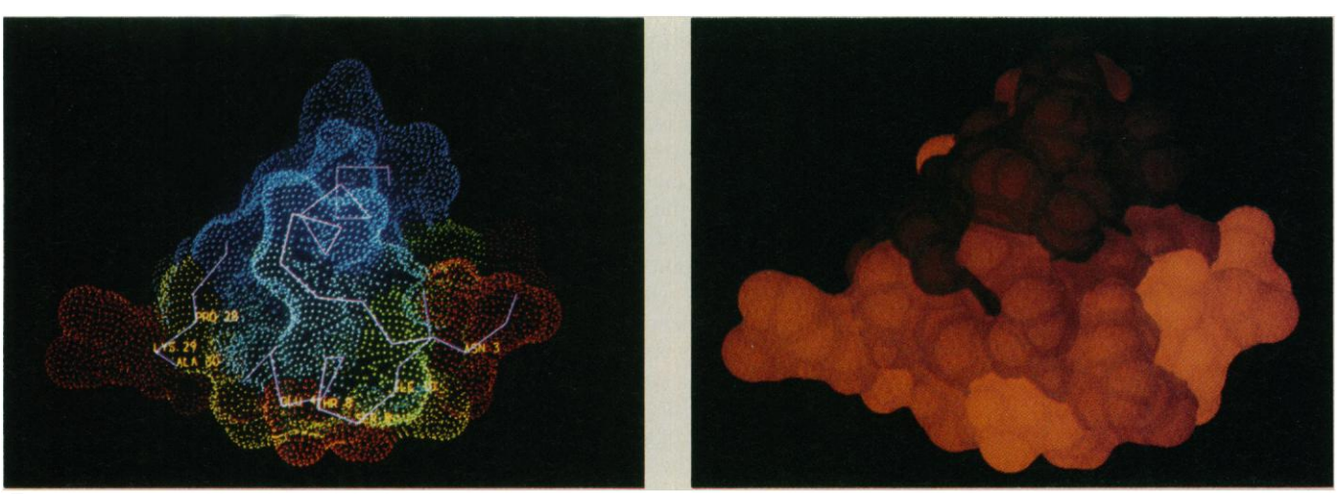
Klug's interest in antigen flexibility and antibody recognition grew out of his laboratory's work on the x-ray crystallography of proteins. Current methods for refining the protein structures derived by x-ray diffraction studies give not only accurate locations for all the atoms in the molecule but also their "temperature factors," which are essentially measures of the atoms' freedom to move. One of the protein structures that Klug and his colleagues have elucidated to high enough resolution to permit the calculation of temperature factors is the coat protein of tobacco mosaic virus (TMV).

Meanwhile, M.H.V. Van Regenmortel and his colleagues at the CNRS Institut de Biologie Moleculaire et Cellulaire in Strasbourg, France, were working to identify those portions of the TMV protein that are recognized by antibodies against it. Some such antigenic determinants, as they are called, consist of short continuous segments of about five to ten amino acids. In others the amino acids may be widely separated in the linear structure of the protein but are close together when it folds.

The Van Regenmortel group identified seven determinants of the continuous type in the TMV protein. "There is a strong correlation between the regions with high temperature factors [indicating the more mobile segments] and the antigenic determinants," Klug says. "Out of the seven, six were regions of high mobility." Although the seventh appeared to be a region of low mobility, it may have been artificially constrained in the crystal where it is in contact with a neighboring molecule. In solution, it might be free to move.

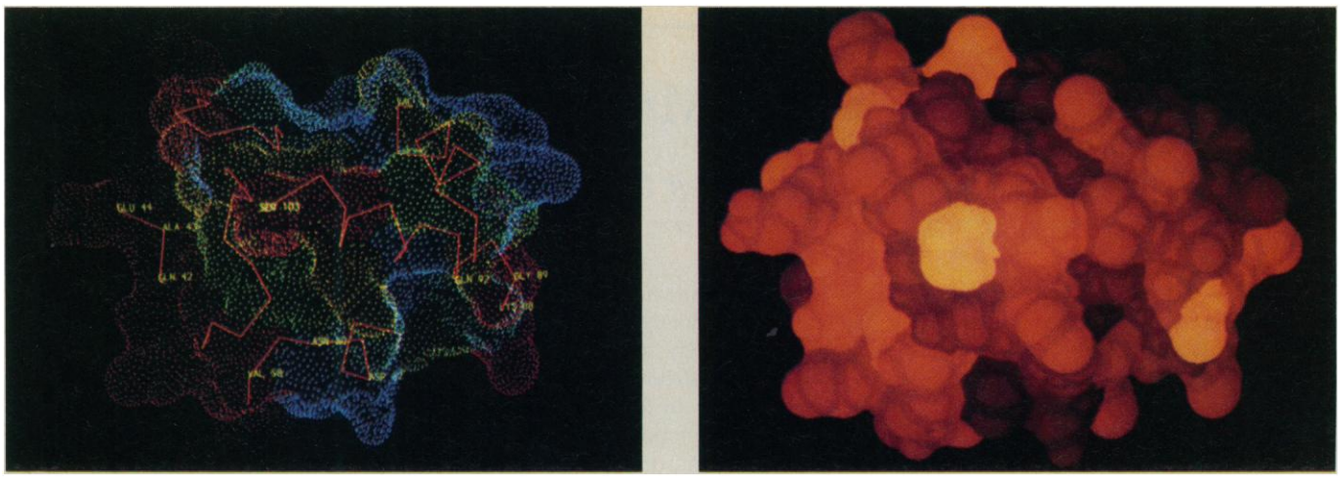
The results thus far apply only to continuous determinants, the investigators point out, and not to the discontinuous type, for which similar analyses are not so readily performed. The movements in question are of the protein backbone, incidentally, not of the amino acid side chains. They probably represent displacements from the mean backbone position of about 1 angstrom.

Lerner and his colleagues took a different route to their finding of a relation between mobility and antibody binding. For the past few years, the Lerner group has been investigating antibodies raised against short, synthetic peptides containing perhaps 10 to 15 amino acids. Such antibodies are often capable of recogniz-



Two computer graphics views of the insulin molecule

In the "glowing coal" model (right) the more mobile segments of insulin have the lightest color. The view to the left shows the molecular surface of insulin with the line indicating the protein backbone. The labeled amino acid residues indicate the antigenic determinants, which are clustered in the more mobile segments (colored red or yellow) of the molecule. [Source: M. L. Connelly, E. D. Getzoff, A. J. Olson, and J. A. Tainer, Research Institute of Scripps Clinic]



Computer graphics views of cytochrome c

As in the case of insulin (see figure on page 819) the antigenic determinants (labeled amino acids) tend to cluster on the more mobile segments of the protein. [Source: M. L. Connelly, E. D. Getzoff, A. J. Olson, and J. A. Tainer, Research Institute of Scripps Clinic]

ing the intact proteins in which the same peptide sequences occur.

The work has opened the door to a possible new way of making vaccines. But it has also created a paradox. "How can you make an antibody to a peptide, which is in the most disordered state, and have the antibody see the cognate sequence in what is supposed to be an ordered state?" Lerner asks. Yet the antibodies did, and with surprisingly high frequency. The Lerner group found in one study that from 25 to 75 percent of monoclonal antibodies to peptides recognize the corresponding native protein. In contrast, there is a good deal of evidence that antibodies to proteins usually react poorly, if at all, with either denatured proteins or peptide fragments.

Conceivably the peptide in solution might assume, for at least a small part of the time, the same conformation that it has in the native protein, thus giving rise occasionally to an antibody that can recognize the protein. However, Lerner does not think that this could explain the high frequency with which monoclonal antibodies to peptides react with proteins. Another possible resolution of the paradox was that the antibodies might recognize those portions of the proteins that have at least some degree of flexibility in their own conformations.

Lerner and his colleagues set out to test this hypothesis on myohemerythrin, an oxygen-carrying protein found in lower animals, including certain marine worms. Wayne Hendrickson of Columbia University and his colleagues have determined the temperature factors for the protein. The Lerner group synthesized 12 peptides, some corresponding to rigid segments of myohemerythrin and others corresponding to mobile segments.

They were able to raise antibodies to every peptide but one. They found that all the antibodies to mobile—"hot" in their terminology—peptides reacted well with myohemerythrin, whereas the antibodies to "cold" peptides did not.

The current results suggest that backbone flexibility is more important in determining where antibodies to proteins bind than either the region's degree of exposure on the protein surface or the hydrophilicity (a measure of compatibility with water) of the constituent amino acids. Both of these characteristics, which are related because hydrophilic residues predominate on protein surfaces, have been used to predict those regions that are likely to be antigenic determinants. But, according to the Klug–Van Regenmortel group, not all exposed regions on the TMV protein are antigenic. Moreover, the hydrophilicity of the amino acids did not correlate with antigenicity nearly as well as mobility did.

Lerner and his Scripps colleagues John Tainer and Elizabeth Getzoff chose myohemerythrin peptides from similarly exposed and hydrophilic regions for their experiments. Exposure and mobility are not necessarily correlated, they note. Peptide segments that have very similar exposed areas in the intact protein can be either hot or cold, but only the peptides from hot regions evoke antibodies that react strongly with the protein.

This does not mean that exposure (or hydrophilicity) has no influence on antigenicity. All things considered, antibodies are more likely to react with the more accessible regions of a protein, although the Lerner group has identified at least one example of a strong antigenic determinant that reacts with antibodies raised against the corresponding peptide even though in the crystal structure it is in a fairly inaccessible region of an influenza virus protein. In solution the molecule can apparently open sufficiently to allow the antibody to have access to the determinant.

A number of other proteins, in addition to the TMV protein and myohemerythrin, show correlations between their antigenic and mobile regions. Both the Klug–Van Regenmortel and Lerner groups, using for the most part published data from a variety of investigators, have found such correlations for lysozyme and myoglobin. The Scripps workers have also found them for hemoglobin, leghemoglobin, cytochrome c, ribonuclease, and insulin. In these cases the antigenic determinants had been located primarily for antibodies raised against the whole protein.

These observations are related to those of other investigators who have often found that antibodies, especially against proteins having functions that have been maintained throughout evolutionary history, usually recognize those portions of the proteins that have changed during evolution and not those that have been conserved. For example, a few years ago, Ronald Jemmerson, who is now at Scripps, and Emanuel Margoliash of Northwestern University noted that this was the case for antibodies against cytochrome c. To explain their observation they proposed that animals preferentially make antibodies to the regions that may vary from species to species because they become tolerant to the conserved regions and fail to recognize them as foreign.

The regions that change are also likely to be the more flexible portions of the molecule, whereas the conserved regions, which carry out the specific functions of the protein and must be maintained in the correct relative positions, are more likely to be rigid. Consequent-

ly, the apparent correlation between antigenicity and mobility may reflect the fact that antibodies are not made to the rigid regions, especially for proteins, such as the hemoglobins and cytochrome c, the functions of which have maintained throughout evolution.

However, myohemerythrin and the coat protein of a plant virus such as TMV should be completely foreign to animals and less likely to be subject to complications from tolerance effects. In fact, Lerner and his colleagues deliberately chose myohemerythrin for this very reason. Nevertheless, they point out that, even if the preferential recognition of flexible areas is a fortuitous result of tolerance, it may still help antibodies carry out their functions by directing them to target areas that are capable of adjusting to give a better fit to antibody structures.

Lerner, Ian Wilson, who is also at Scripps, and their colleagues plan to further test the hypothesis that mobility is an important factor in antibody binding in an experiment to be conducted with Sydney Brenner, who is at the MRC's Cambridge Laboratory. Brenner has suggested another possible explanation for the observation that antibodies to peptides so frequently recognize the intact proteins. Because the immune system evolved to recognize proteins, he suggests, it may be fundamentally biased to produce antibodies that recognize conformations that actually exist in proteins.

The investigators have recently performed a computer search to identify a set of peptide sequences, containing at least a half-dozen or so amino acids that occur in unrelated proteins. The idea is to make antibodies to the peptides and see whether they can recognize the proteins that contain the peptides. If Lerner is correct, an antibody to a given peptide may recognize different proteins that contain the peptide even though its conformation may vary among them. But if Brenner is correct, an antibody should bind to different proteins only if the peptide segment has the same conformation in all of them.

Whether other types of interactions between proteins also involve binding to flexible sites is unclear. Lerner notes that there is a fundamental difference between antigen-antibody interactions and those between enzymes and their substrates or hormones and their receptors. "Enzymes have been honed over millions of years of evolution to do one thing," he says. "The immune system has evolved to handle all comers."

Mobility in the form of the relatively large conformational changes that play a role in enzyme activation or inhibition is well known. But the type of mobility implicated in antibody-antigen recognition is much more localized and restricted in scope. Pinpointing such subtle changes when protein substrates interact with their enzymes may be difficult. So far the x-ray crystallographers have solved the three-dimensional structures of only about 25 proteins to a resolution sufficiently high to calculate temperature factors. X-ray diffraction studies of pairs of interacting proteins may also help locate local mobility changes but these are at a very early stage of development. However, antibodies themselves may prove useful as probes of backbone flexibility. Arthur Olson of Scripps asserts, "Immunology is turning out to be one of the tools for exploring mobility." Modern nuclear magnetic resonance methods may also help in this regard.

Finally, the mobility work may facilitate the development of vaccines from synthetic peptides by helping in the selection of those peptides that elicit antibodies that react strongly with the parent protein. The main problem at the moment is the small number of proteins for which temperature factors can be calculated. These do not include proteins of pathogens for which researchers are trying to devise new vaccines. Meanwhile, Klug suggests, selecting peptides from the loops or turns of the target molecule, which are likely to be mobile, may prove more helpful than relying on other possible indicators of antigenicity such as hydrophilicity.—**JEAN L. MARX**

Additional Reading

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2. J. A. Tainer, E. D. Getzoff, H. Alexander, R. A. Houghton, A. J. Olson, R. A. Lerner, W. A. Hendrickson, *ibid.* **312**, 127 (1984).
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Soft X-ray Laser at Lawrence Livermore Lab

An extensive battery of diagnostic tests makes Livermore's the first well-documented x-ray laser; Princeton is not far behind

Researchers from the Lawrence Livermore National Laboratory have claimed success in the long quest to build an x-ray laser. The group made its announcement on 29 October, on the opening day of the American Physical Society's Division of Plasma Physics meeting in Boston. Laser scientists who have examined the Livermore experiments enthusiastically concur with the claim. "There is no doubt at all that they have a laser," says laser pioneer Charles H. Townes of the University of California at Berkeley.

Livermore's laser emitted radiation at 206 and 209 angstroms, with a maximum intensity about 700 times that expected for spontaneous emission (fluorescence) at the same wavelengths in the highly ionized selenium plasma that served as the laser medium. Some physicists prefer the designation extreme ultraviolet (XUV) for this wavelength range.

At the same meeting, scientists from the Princeton Plasma Physics Laboratory reported amplification by a factor of 100 of 182-angstrom radiation from a hydrogen-like carbon plasma. However, Szymon Suckewer, head of the Princeton group, says he would like to see an enhancement of intensity of 1000 before claiming a laser. "Livermore is already there," he adds.

Diagnostics is the strength of the claim of the 27-member Livermore group, which is headed by Dennis Matthews. Previous assertions of x-ray lasing either have been refuted or lie in limbo for lack of corroborating evidence. "Livermore has done exquisitely careful diagnostic tests," says Stephen Harris of Stanford University, who is working on his own version of an XUV laser. Both Harris and Townes are members of a select group of laser experts called in by Livermore to go over the x-ray laser experiment with a fine-tooth comb prior to making a public announcement.

Careful diagnostics are needed because, comparatively speaking, all candidate x-ray lasers have had rather feeble