Reports

Mechanisms of Antibody Binding to a Protein

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The mechanisms of antibody binding to a protein were studied by an analysis of specific amino acid residues critical to nine antigenic sites on myohemerythrin. Rabbit antisera to the whole protein were assayed for binding to more than 1500 distinct peptide analogs differing from the protein sequence by single amino acid replacements. The results, combined with information from the three-dimensional crystallographic structure, were used to evaluate probable mechanisms of antibody binding at individual sites. The data from all sites examined indicate that initial binding to solvent-exposed amino acid residues may promote local side-chain displacements and thereby allow the participation of other, previously buried, residues.

ANTIGENIC SITES ON THE PROTEIN myohemerythrin (MHr) were chosen from antigenicity profiles of seven rabbit antisera produced against the whole protein (anti-MHr) and mapped with the use of 113 overlapping hexapeptide homologs of the MHr sequence (*1*). These sites (referred to by inclusive amino acid residue numbers) represent peaks of frequent antigenicity, as probed with peptides, as well as one peptide (residues 16 to 21) recognized with high titer by a single antiserum. The roles of individual side chains in each site were characterized by measuring the reactivities of appropriate antisera with sets of 120 peptides that included all possible analogs (replacement nets) differing in sequence from the parent peptide by replacement of a single amino acid residue (Fig. 1). Competition data measured in a solution phase assay show that native MHr inhibits the anti-MHr binding of peptides corresponding to the antigenic sites examined (*1*). This competition suggests that the antibody binding role for an individual side chain in the protein can be inferred from its role in the peptide.

Such replacement net data made it possible to assign each residue of a peptide to one of the following four categories (Table 1): essential, selected, partially replaceable, or generally replaceable. In site 4 to 9 (Fig. 1A), antigenic reactivity was lost when replacing Glu^6 or Tyr^8 with any of the other 19 commonly occurring amino acids; thus these residues are classified as essential to antibody binding. This ability of antibodies to distinguish single amino acid changes in antigens has also been shown in fine specificity studies of other systems (*2, 3*). In contrast to the required specificity of Glu^6 and Tyr^8, Ile^4 can be replaced by all common amino acids without affecting peptide binding by the antiserum (generally replaceable). Likewise, Pro^5 is replaced by most other amino acids without influencing antibody binding; Pro^7, however, is partially replaceable and Val^9 is selected (selectively replaceable only by Ala, Ile, Ser, or Thr). In some cases, replacement net data are only partially interpretable and may reflect the existence of multiple overlapping antigenic sites recognized by polyclonal antisera. For example, replacement nets in which a given level of reactivity is partially restored by many substitutions at a given position and completely restored by a few, seem to indicate that two overlapping sites are present, one in which that residue is essential or selected, and another in which it is generally replaceable. Overlapping antigenic determinants have also been indicated by experiments examining the repertoire of monoclonal antibody specificities to protein antigens (*4*).

Residues identified as critical (that is, essential or selected) to interaction with anti-MHr from one rabbit are usually critical to interaction with polyclonal antibodies from other responding rabbits (Table 1). This applies both within the same peptide (for example, Phe^{80} and Lys^{83} in site 80 to 85) and between overlapping peptides (Tyr^{67}, Glu^{69}, and Val^{70} in sites 63 to 68, 65 to 70, and 68 to 72). Using monoclonal antibodies, another investigator (*5*) has identified within the C helix of MHr the same sites and patterns of critical residues. Thus, intrinsic stereochemical properties of specific residues within the three-dimensional environment of a protein bias the selection of the critical residues recognized by the immune system, at least for antigenic sites probed by peptides. Although MHr antisera from different individual animals may recognize different sites, the chemical basis for interaction at a given recognized site appears to be similar among the applicable rabbit antisera.

As is shown in Table 2, eight of the nine sites each include one or more highly exposed and accessible critical side chains (>55 percent exposed side-chain area, accessible to an antibody binding domain as modeled by a 15 Å radius sphere), and one or more largely buried and inaccessible critical side chains (≤5 percent exposed side-chain area, nearly or totally inaccessible to a solvent-sized probe of 1.4 Å radius) within the native protein structure. Moreover, critical, buried side chains seem to be directly involved in binding to the antibody, rather than simply stabilizing an appropriate conformation of the peptide, although conformational stabilization cannot be definitively ruled out. First, short peptides are very unlikely to have fixed or strongly favored conformations in solution and would be expected to adapt to the more structured antibody binding site (*6*). Second, often only one amino acid type at a given position permits binding, rather than a set of acceptable amino acid replacements with similar properties (Fig. 1), as would be expected for side chains performing a conformational role. Third, buried critical residues are not randomly located, but show a specific positional relation in the protein structure to the solvent-exposed critical residues, which is most frequently seen as a gating phenomenon (see below). This proposed antibody binding role of buried hydrophobic side chains agrees with the analysis of known protein complexes in which binding affinity depends in part on maximizing the hydrophobic surface area in the buried interface (*7*), with the previously noted importance of hydrophobic and aromatic residues in antigenicity (*8*), and with the prevalence of complementary hydrophobic and aromatic residues in the binding sites of antibodies (*3, 9*).

The critical residues within the characterized sites form microassemblies: three-dimensional clusters of closely interacting side chains (Fig. 2). A network of microassemblies interconnects to form the backbone of the most frequently reactive sites (*1*). From this work, three levels of structural hierarchy can be proposed within a protein antigen: (i) microassemblies of sequence-local, criti-

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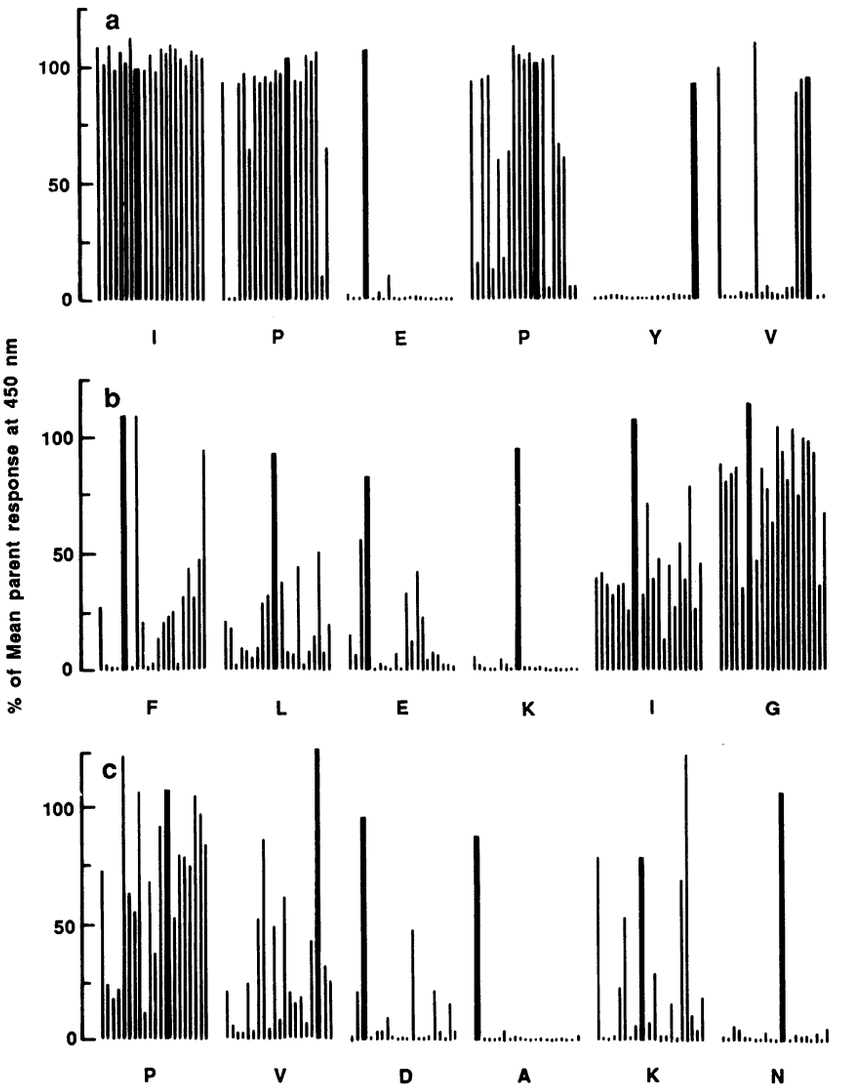
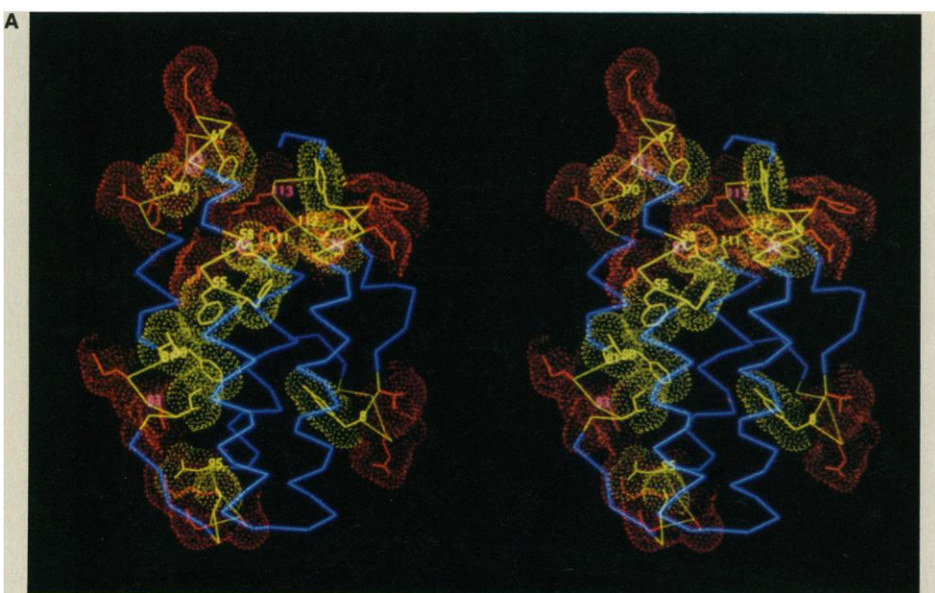


Fig. 1. Replacement nets identifying the MHr residues essential for antibody binding to peptide homologs. (**a**) Site 4 to 9, Ile-Pro-Glu-Pro-Tyr-Val, antiserum 7. (**b**) Site 80 to 85, Phe-Leu-Glu-Lys-Ile-Gly, antiserum 7. (**c**) Site 90 to 95, Pro-Val-Asp-Ala-Lys-Asn, antiserum 6. ELISA (enzyme-linked immunosorbent) absorbances for each hexapeptide, measuring the reactivity with antibodies to MHr, are represented as a percentage of the mean absorbance of the six replicates of the parent peptide (thicker bars). Each block of bars represents 20 hexapeptides that differ from the parent peptide by replacement of the amino acid residue indicated underneath. The identity of each substituted residue (one of the 20 genetically coded amino acids) is indicated by the position of the individual bar within each block, based on alphabetical order of the single-letter code for the amino acids: 1A (Ala), 2C (Cys), 3D (Asp), 4E (Glu), 5F (Phe), 6G (Gly), 7H (His), 8I (Ile), 9K (Lys), 10L (Leu), 11M (Met), 12N (Asn), 13P (Pro), 14Q (Gln), 15R (Arg), 16S (Ser), 17T (Thr), 18V (Val), 19W (Trp), 20Y (Tyr). Thus, the leftmost bar of Fig. 1A represents peptide APEPYV and the rightmost bar represents peptide IPEPYY. In site 4 to 9 (A) Glu^6 and Tyr^8 are essential and Val^9 is selected (selectively replaceable). In site 80 to 85 (B) Lys^{83} is essential and Phe^{80}, Leu^{81}, Glu^{82}, and Ile^{84} are selected. In site 90 to 95 (C) Ala^{93} and Asn^{95} are essential and Asp^{92} is selected. The experimental methods used to obtain replacement net data including appropriate controls and scaling have been described (*1, 25*).



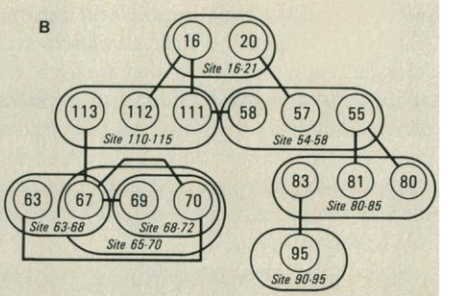


Fig. 2. Connectivity of the essential residues. (**A**) Stereo computer graphic overview showing the microassemblies of critical side chains and their interactions. Bonds (shown as lines) and molecular surface (dots) are shown for the critical residues in the nine sites identified. The Cα backbone is blue, the bonds and individual surfaces of buried critical side chains are yellow, and the bonds and exposed surface of solvent-exposed critical side chains are red. All residues identified by sequence number at the Cα position participate in interactions between sites, identified schematically in (B) except for labeled residue 8, which marks the single site not involved in interlocking interactions. The orientation is similar to that shown in figure 2C of Geysen *et al.* (*1*), where computer graphics methods are also described. (**B**) Schematic drawing illustrating the connectivity of the critical residues (circled) identified in eight sites (ellipses). Connecting lines indicate the nonbonded van der Waals contacts (identified by contact distances and individual atomic radii), charge interactions, and hydrogen bonds (≤3 Å from hydrogen donor to hydrogen acceptor).

cal, side chains forming partial or complete determinants for specific binding, (ii) discontiguous conformational antigenic sites formed from adjoining microassemblies, and (iii) superassemblies of antigenic sites (*1*), which may define the chemical and conformational environment favored for antigenic recognition. This hierarchy concurs with previous observations concerning the clustering of determinants to form large antigenic surfaces (*10*).

The stereochemical relations of MHr critical residues identified by means of peptide analogs suggest that specific recognition of highly exposed side chains is coupled to movements, thus allowing antibody interaction with additional, buried, usually hydrophobic side chains. Here we combine antigenic and structural data to show in detail one example of a proposed antibody-protein interaction mechanism involving recognition, induced fit, and binding. (Other examples are given in Fig. 3.) Site 4 to 9 includes essential residues Glu^6, Tyr^8, and selected residue Val^9. The direct interaction of the incoming antibody with Glu^6 can be envisioned to break the charge-charge interaction between Glu^6 and Lys^{100} (an indirect salt bridge through a bound water molecule). The resultant disruption of close packing between the hydrophobic portions of the Tyr^8 and Lys^{100} side chains, and of the hydrogen bond between the Tyr^8 hydroxyl group and the main chain of Lys^{100}, could allow the exposure of underlying, buried Tyr^8 (Fig. 3A). An antibody positioned to simultaneously recognize the side chains of Glu^6, Val^9, and Tyr^8 (after rotation about the Cα–Cβ bond to allow accessibility) would also contact Pro^7. Substitution with large aromatic side chains at this position eliminates antibody binding, presumably by steric hindrance.

In all nine sites examined in detail, the antibody can be inferred to bind to one or more solvent-exposed residues in the protein, allowing local side-chain rearrangements that may uncover previously buried atoms of the protein antigen. Antibody interaction with critical buried residues in one site may help induce side-chain adjustments in adjoining sites, making the binding of additional antibodies more favorable (*11*), and thus cooperative. In some cases, the extent of induced fit inferred is limited to minor movements of solvent-exposed side chains (sites 63 to 65, 65 to 70, 68 to 72, and 90 to 95), similar to those seen in molecular dynamics calculations or among different crystalline forms of the same protein molecule (*12*). These subtle localized changes do not alter the overall tertiary folding of the protein and may fall within the deviations (*3*) between the free and antibody-complexed crystal forms of lysozyme (root-mean-square deviations of 0.64 Å for Cα positions coupled with some larger differences in side-chain conformation). For other sites, additional side-chain rotations of buried residues in the presence of the antibody interface (sites 4 to 9, 16 to 21, 54 to 58, and 80 to 85), or some small displacements of main-chain atoms (site 110 to 115) may be required. All of these local displacements implicated in antigenic recognition affect the surface topography of the protein antigen (Fig. 4). If such rearrangements occur only for low affinity antibodies capable of recognizing linear peptides, they are biologically unimportant. To test this, antibody affinities for native MHr were measured (*13*) by using antibodies raised against MHr and selected by affinity purification against peptides 3 to 16, 37 to 46, 63 to 72, or 73 to 82. The affinity constants derived by standard Scatchard analysis using immune precipitation methods (*13*) all fell in the range of 10^7 to 10^8, which are about average for antibodies raised against proteins (*14*). Based upon this result, and competition by native MHr in solution for all the sites examined, the reactivities of the antibodies for the identified MHr sites appear to be biologically relevant.

Antibodies share with other proteins the ability to bind with high affinities to small molecules (*14*), presumably without requiring significant conformational change. The protein avidin binds the smaller molecule biotin with an association constant of 10^{15}, representing about 20 kcal/mol (*15*). However, the considerably larger interface formed by an antibody-protein complex may be able to provide sufficient binding energy to pay for local rearrangements, while maintaining or even improving high affinity. Assuming that each of the 748 $Å^2$ of protein surface buried by the antibody in the lysozyme-antibody complex (*3*) contributes 20 cal/mol (*16*) of binding energy, the experimental association constant of 4.5×10^7 (equivalent to 10.4 kcal/mol) leaves about 4.5 kcal/mol remaining for induced fit. Although the individual energetic components for antibody binding to sites in MHr are not known, the antibody affinities (10^7 to 10^8) associated with the energy remaining after any rearrangements (more than 10 kcal/mol) correspond to a significant portion of the observed net sta-

Table 1. Critical residues for antibody binding. Each site in MHr is named by the residue number range and amino acid sequence in one-letter code. The nine sites (including three overlapping sites from residues 63 to 72) were chosen for having frequent reactivity or high titer (or both), as assayed by reaction of anti-MHr with peptide homologs that compete with native MHr for binding. The details of titer measurements, competition assays, and identification of antisera have been described (*1*). The type of replaceability for the different antisera tested is given under the three-letter amino acid code as *, essential; (*), selected; (r), partially replaceable; and r, generally replaceable. Sites 40 to 45 (SAPNLA) and 88 to 93 (SAPVDA) were also tested, but were excluded because of their sequence homology. The set of critical residues for each of the other sites maps to a single MHr location.

Site	Sequence	Antiserum	Titer	Class of replaceability					
				Ile	Pro	Glu	Pro	Tyr	Val
4–9	IPEPYV	7	>2000	r	r	*	(r)	*	(*)
				Val	Phe	Tyr	Glu	Gln	Leu
16–21	VFYEQL	5	>2000	(*)	*	*	*	*	r
				His	Phe	Thr	His	Glu	
54–58	HFTHE	4	1020	*	*	*	*	(*)	
		5	1530	*	*	(*)	(*)	(*)	
		6	1970	*	*	*	(r)	(*)	
		7	480	*	*	(*)	(*)	(*)	
				Asp	Ala	Ala	Lys	Tyr	Ser
63–68	DAAKYS	4	>2000	*	*	(r)	*	*	(r)
				Ala	Lys	Tyr	Ser	Glu	Val
65–70	AKYSEV	6	>2000	r	(r)	*	r	*	(*)
		7	>2000	r	(*)	*	(r)	*	(*)
				Ser	Glu	Val	Val	Pro	
68–72	SEVVP	5	890	(r)	*	*	*	*	
				Phe	Leu	Glu	Lys	Ile	Gly
80–85	FLEKIG	4	>2000	*	(*)	*	*	*	r
		5	>2000	*	(*)	(*)	*	*	(*)
		6	830	*	*	r	*	(*)	r
		7	1900	(*)	(*)	(*)	*	(*)	r
				Pro	Val	Asp	Ala	Lys	Asn
90–95	PVDAKN	4	580	r	r	*	*	(*)	*
		5	1600	(r)	(*)	*	*	(r)	*
		6	>2000	r	(r)	(*)	*	(r)	*
				Thr	Asp	Phe	Lys	Tyr	Lys
110–115	TDFKYK	7	930	r	(*)	*	*	(r)	r

bility for a protein (about 16 kcal/mol) (*15*). Antibodies, as well as protein antigens, may undergo local side-chain adjustments: crystallographic studies of light chain dimers have revealed rotations and translations of aromatic side chains, and small displacements of the three hypervariable loops to improve complementarity with bound peptide ligands (*17*). Subtle, localized changes at antigen-antibody interfaces may actually improve stereochemical complementarity, and hence affinity, by reducing gaps in the interface (costing about 1.6 kcal per water molecule volume) (*18*), or by forming hydrogen bonds (0.5 to 1.0 kcal/mol, uncharged, to 3.5 to 4.5 kcal, charged) (*19*).

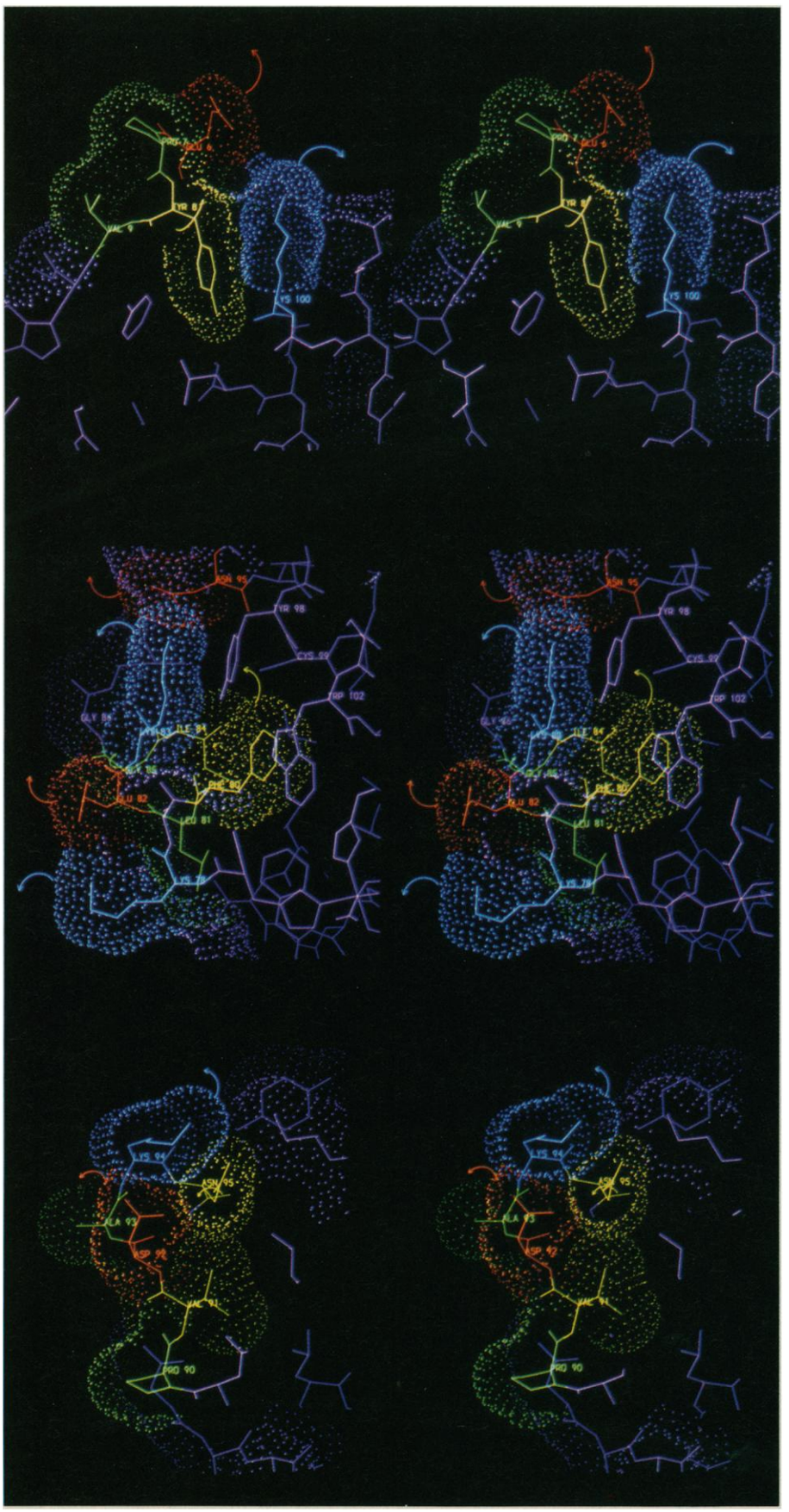
Antigen and antibody proteins are stable in solution, so that large (energetically costly) conformational changes during complex formation are unlikely. As verified by the 2.8 Å resolution structure of the lysozyme-antibody complex (*3*), antibodies do not need to unfold proteins in order to bind. Yet, protein surfaces are inherently flexible and protein-protein interactions commonly elicit subtle, but nevertheless interesting, atomic shifts (ranging from less than 1 Å to more than 10 Å) that are important for complex stabilization (*15, 20*). In a more extreme example, the transition from the 4 Zn to the 2 Zn form of insulin changes the conformation of eight residues from α helical to extended conformation (*21*) requiring atomic shifts of up to 21 Å. On the other hand, data from protein crystal structures of site-directed mutants indicate that very small changes (≤1 Å) in side-chain position and solvation can have substantial effects on binding affinity (*22*).

Antibodies are uniquely successful in recognizing and forming stable complexes with

Table 2. Properties of MHr residues critical to antibody binding of peptide homologs. Critical residues are labeled by residue number and three-letter amino acid code. Secondary structure is taken from the crystallographic structure of MHr (*26*). Exposed side-chain area for hydrophobic (carbon), hydrophilic (noncarbon), and all side-chain surface area exposed in the context of the protein structure is expressed as a percentage of the total side-chain surface area in the absence of neighboring protein residues (*1*). Packing density was determined numerically as the surface area buried in noncovalently bonded close-packing; a modified version (*1*) of the program MS (*27*) was used. Sphere accessibility was determined analytically as the radius of the largest spherical probe that has access to a given side-chain without collision or interpenetration with the surface from any other residue (*28*). Sphere radii of zero represent side chains inaccessible to a water-sized (1.4 Å radius) probe sphere and sphere radii ≥15 were evaluated as 15Å (roughly the radius of an immunoglobulin binding domain).

Residue identity	Secondary structure	Percent of exposed side-chain area			Packing density (Å)	Sphere accessability radius (Å)
		Hydrophobic	Hydrophilic	All		
6 Glu	Loop	81	70	77	32	15
8 Tyr	Loop	0	0	0	140	2
9 Val	Loop	49	0	49	67	15
16 Val	Loop	0	0	0	96	0
17 Phe	Loop	56	0	56	63	15
18 Tyr	Loop	22	10	19	98	15
19 Glu	Loop	81	32	63	33	15
20 Gln	A helix	46	89	70	35	15
54 His	B helix	0	0	0	92	1
55 Phe	B helix	2	0	2	120	2
56 Thr	B helix	75	57	70	36	15
57 His	B helix	26	36	29	78	15
58 Glu	B helix	0	0	0	65	0
63 Asp	Turn	54	25	39	49	15
64 Ala	Turn	66	0	66	21	15
66 Lys	Turn	79	95	85	33	15
67 Tyr	Turn	5	3	5	132	2
69 Glu	Loop	34	60	44	61	15
70 Val	Loop	16	0	16	77	8
71 Val	C helix	60	0	60	39	15
72 Pro	C helix	44	0	44	35	15
80 Phe	C helix	0	0	0	122	0
81 Leu	C helix	26	0	26	78	8
82 Glu	C helix	61	57	59	46	15
83 Lys	C helix	25	35	29	80	15
84 Ile	C helix	0	0	0	105	0
85 Gly	C helix	–	–	–	23	13
91 Val	Loop	0	0	0	102	1
92 Asp	Loop	83	28	54	37	15
93 Ala	Loop	94	0	94	7	15
94 Lys	Loop	68	59	65	46	15
95 Asn	D helix	0	16	11	72	11
111 Asp	D helix	0	0	0	46	0
112 Phe	D helix	19	0	19	106	15
113 Lys	Loop	50	87	64	71	15

Fig. 3. Mechanisms inferred from high resolution structural and immunological data for the three sites characterized in Fig. 1. These stereo pairs represent slices through the MHr atomic model (isolated by clipping planes) as displayed on computer graphics. Outside the site, bonds and molecular surface are colored purple. Within each antigenic site, bonds and surface are colored blue for positively charged, exposed, critical side chains, red for negatively charged, exposed, critical side chains, yellow for bonds and individually surfaced side chains of critical buried residues, and green for noncritical residues. Curved arrows indicate plausible side-chain movements when antibody binding to critical exposed polar side chains results in the breaking of one or more charged and/or hydrogen bonding interactions. (**A**) The structural interactions for site 4 to 9, Ile-Pro-Glu-Pro-Tyr-Val, of the NH_2-terminal loop. Binding of the exposed Glu^6 side chain (red, top center) could disrupt the interaction with the Lys^{100} side chain (blue, right) which packs against and forms a gate to the surface for the buried Tyr^8 side chain (yellow, center). (**B**) Site 80 to 85, Phe-Leu-Glu-Lys-Ile-Gly, located in the C helix, has an apparent interaction mechanism that involves concerted movements of several side chains. The critical exposed residues, Glu^{82} (red, left center) and Lys^{83} (blue, left center), participate in a stabilizing salt bridge (Glu^{82}-Lys^{78}, lower left) across a turn of helix and a charged hydrogen bond (Lys^{83}-Asn^{95}, upper left) between helices that may be disrupted by antibody binding. Critical, buried residues Phe^{80} and Ile^{84} (yellow, center) are in van der Waals contact with each other, and rotations of their side chains do not easily allow their antigenic accessibility without additional concerted movements of other side chains. However, binding of essential Lys^{83} by an antibody might break the hydrogen bond to Asn^{95}, weakening the interaction between the C and D helices, and potentially exposing parts of the buried side chains of Phe^{80} and Ile^{84}. Antibody binding to Glu^{82}, which is critical to most antisera (Table 1), might break the intrahelical Glu^{82}-Lys^{78} salt bridge and increase the conformational freedom provided by Gly^{85} and Gly^{86} (left center) at the end of the C helix. (**C**) In site 90 to 95, Pro-Val-Asp-Ala-Lys-Asn, solvent-exposed Asp^{92} (red) and Ala^{93} (green, far left), and mostly buried Asn^{95} (yellow) are critical to antibody recognition in all antisera examined. Solvent-exposed Lys^{94} (blue, upper left) and buried Val^{91} (yellow, lower center) are also selected in specific antisera. The side chain of Asn^{95} (top center) makes charged hydrogen bonds to both Asp^{92} (below left) and Lys^{94} (above left) within the same epitope and to Lys^{83} in adjoining site 80 to 85. Antibody binding to Asn^{95} (or to Lys^{83}) could thus affect both sites. The side chain of Asn^{95} is mostly buried underneath Asp^{92} and Lys^{94} (only the terminal amino group is solvent-exposed) and might become more accessible upon antibody binding to Asp^{92} or rearrangements of the mobile side chain of Lys^{94}. Thus only minor, local, side-chain, movements of the antigen appear to be required to allow interaction with the antibody.



many different protein sites. Our work suggests that to do this antibodies may adapt the exposed molecular surface of the antigen to resemble buried interfaces in stable protein-protein complexes. Some immunodominant regions may already form good interfaces, otherwise the antibody binding energy must be sufficient to absorb the cost of any localized side-chain rearrangement. Antibody binding may locally develop optimal intermolecular complementarity by disrupting surface-exposed salt bridges and hydrogen bonds, by promoting the entropically favored removal of the bound water molecules that stabilize the polar side-chain positions and surface topography of the free antigen, and by increasing the contribution of hydrophobic side chains in the interface. These mechanisms are consistent with previously observed antigenic biases for high mobility and convex shape (*18, 23*), which may influence the process of induced fit in different ways, with shape potentially domi-

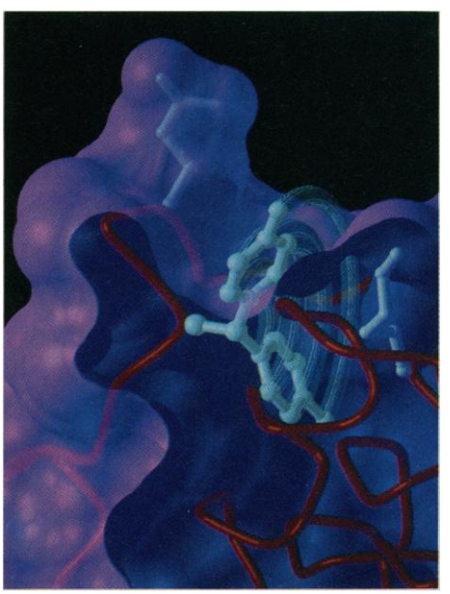


Fig. 4. Induced fit and antigenic surface topography. The MHr exposed molecular surface (shown transparent purple) is sliced away to reveal its inner surface (deep blue) and alpha-helical backbone (red tube). The stereochemistry of critical residues suggests a mechanism of induced fit involving side-chain movements to break the original interaction between surface-exposed Glu^{6} (light blue bonds, upper left) and Lys^{100} (light blue bonds, right) and allow previously buried Tyr^{6} side chain (center, blue bonds shown in two positions) to rotate out from the buried native position (lower) and interact with the antibody molecule (not shown). Thus, in the proposed mechanism of interaction, antigenic recognition causes bond rotations that change the surface topography of the antigenic site. [Rendered by M. Pique on CONVEX and SUN computers using MCS (*27*) and the Dicomed film writer at the San Diego Supercomputer Center]

nating the kinetics of water release and mobility helping to reduce the energetic costs of correlated, local, side-chain movements (*1*). In effect, the proposed mechanisms of antigen-antibody union show striking similarities to the induced fit mechanisms often implied in enzyme-substrate (*15, 20*) and DNA-protein interactions (*24*).

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The Resonating Valence Bond State in La_2CuO_4 and Superconductivity

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The oxide superconductors, particularly those recently discovered that are based on La_2CuO_4, have a set of peculiarities that suggest a common, unique mechanism: they tend in every case to occur near a metal-insulator transition into an odd-electron insulator with peculiar magnetic properties. This insulating phase is proposed to be the long-sought "resonating-valence-bond" state or "quantum spin liquid" hypothesized in 1973. This insulating magnetic phase is favored by low spin, low dimensionality, and magnetic frustration. The preexisting magnetic singlet pairs of the insulating state become charged superconducting pairs when the insulator is doped sufficiently strongly. The mechanism for superconductivity is hence predominantly electronic and magnetic, although weak phonon interactions may favor the state. Many unusual properties are predicted, especially of the insulating state.

RECENTLY HIGH-TEMPERATURE SUperconductivity has been observed in a number of doped lanthanum copper oxides near a metal-insulator transition (*1*), a pattern exhibited previously by $(Ba,Pb)BiO_3$ (*2*). The crystal structure suggests that the Cu^{2+} is in an $S = 1/2$, orbitally nondegenerate state, strongly hybridized with the surrounding oxygen *p*-levels, and this is in agreement with high-temperature magnetic data (*3*) on the stoichiometric, insulating compound La_2CuO_4.

The appropriate model seems to be the basic nearly half-filled Hubbard model (*4*) with moderately large repulsion energy U and antiferromagnetic exchange constant $J = t^2/U$ where t is the site-hopping matrix element. The K_2NiF_4 structure is a well-known case in which the magnetic layers are relatively weakly interacting, and in the temperature range 30 to 70 K we can assume magnetic two-dimensionality. This led me to reexamine the idea of the "resonating valence-bond" (RVB) state (*5*).

Early doubts about the nature of the ground state of the antiferromagnetic Heisenberg Hamiltonian

$$H = J\sum_{innj} \vec{s}_i \cdot \vec{s}_j \qquad (1)$$

of Hulthén (*6*) and Marshall (*7*) (where $\vec{s}_i$ is the spin at site i and $innj$ indicates summation over nearest neighbors i and j) seemed to have been laid to rest by arguments from quantum fluctuations of spin waves in the Néel state (*8*) in >1 dimension, and by experimental observations of antiferromagnetism. In 1973, however, Anderson (*5*) proposed that, at least in the triangular two-dimensional antiferromagnet for $S = 1/2$, and perhaps in other cases, the ground state might be the analog of the precise singlet in the Bethe solution of the linear antiferromagnetic chain (*6*). In both cases, the zeroth order energy of a state consisting purely of nearest neighbor singlet pairs is more nearly realistic than that of the Néel state, and I

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