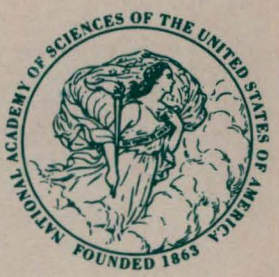
AUGUST 1989
VOLUME 86
NUMBER 15



Proceedings
OF THE
National Academy of Sciences
OF THE UNITED STATES OF AMERICA

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Editorial correspondence: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, 2101 Constitution Avenue, Washington, DC 20418.

Business correspondence: Circulation Office of the PROCEEDINGS, National Academy of Sciences, 2101 Constitution Avenue, Washington, DC 20418.

Information for Contributors: See p. i (of this issue) and pp. i–viii of issue number 1, January 1989.

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Back Issues: Volumes 81–85, January 1984 and thereafter, are available from the Circulation Office of the PROCEEDINGS. The price of a single issue is $20.00.

Second class postage paid at Washington, DC, and at additional mailing offices. PRINTED IN THE USA

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (ISSN-0027-8424) *is published semimonthly by* THE NATIONAL ACADEMY OF SCIENCES, *2101 Constitution Avenue, Washington, DC 20418.*

POSTMASTER: Send address changes to: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 2101 Constitution Ave., Washington, DC 20418.

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5938–5942, August 1989
Immunology

Structure of an antibody–antigen complex: Crystal structure of the HyHEL-10 Fab–lysozyme complex

(x-ray crystallography/complementarity/discontinuous epitope)

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Contributed by David R. Davies, April 24, 1989

ABSTRACT **The crystal structure of the complex of the anti-lysozyme HyHEL-10 Fab and hen egg white lysozyme has been determined to a nominal resolution of 3.0 Å. The antigenic determinant (epitope) on the lysozyme is discontinuous, consisting of residues from four different regions of the linear sequence. It consists of the exposed residues of an α-helix together with surrounding amino acids. The epitope crosses the active-site cleft and includes a tryptophan located within this cleft. The combining site of the antibody is mostly flat with a protuberance made up of two tyrosines that penetrate the cleft. All six complementarity-determining regions of the Fab contribute at least one residue to the binding; one residue from the framework is also in contact with the lysozyme. The contacting residues on the antibody contain a disproportionate number of aromatic side chains. The antibody–antigen contact mainly involves hydrogen bonds and van der Waals interactions; there is one ion-pair interaction but it is weak.**

The interaction of antibodies with protein antigens has been the subject of several recent crystallographic investigations. These include complexes of hen egg white lysozyme with the Fab fragments of the monoclonal anti-lysozymes D1.3 (1) and HyHEL-5 (2) and a Fab complex with influenza neuraminidase (3). From these data a common pattern of interaction is emerging (4) in which there is a high degree of complementarity between the interacting surfaces of the antibody and antigen; the epitope is made up of several small, discrete segments of the polypeptide chain; and relatively small conformational changes occur in the antigen as a result of binding. Here we report the x-ray analysis of HyHEL-10 Fab–lysozyme, in which the antigenic site differs from the two previous examples. The results complement the previous studies but differ from them in several ways.

HyHEL-10 is an IgG1(κ) antibody specific for hen egg white lysozyme. The affinity of HyHEL-10 for hen egg white lysozyme, as estimated by PEG immunoprecipitation, is 1.5 × 10^9 M^{-1} (M. E. Denton and H. A. Scheraga, personal communication), slightly lower than that of HyHEL-5, thus making HyHEL-10 intermediate in affinity between HyHEL-5 and D1.3.§

HyHEL-10 expresses a member of the V_H*36-60* variable gene segment family, the *DQ52* diversity gene segment, and the J_H*3* joining gene segment in the heavy (H) chain and a $V_κ$*23* gene and $J_κ$*2* in the light (L) chain (9). Thus, HyHEL-10 is structurally distinct from HyHEL-5 (which expresses V_H*J558* and $V_κ$*4*) and D1.3 (which expresses V_H*Q52* and $V_κ$*12/13*).

The publication costs of this article were defrayed in part by page charge

MATERIALS AND METHODS

Crystals of the complex of HyHEL-10 Fab with hen egg white lysozyme, grown as described (11), exhibit the symmetry of space group $P2_12_12_1$ with a = 57.47, b = 118.73, c = 137.68 Å and one Fab–lysozyme complex per asymmetric unit.

Intensity data were collected with the Mark II multiwire detector system at the University of California, San Diego (12). The R factor relating the intensities of symmetry-related reflections (12) was 0.066. The data set used in the structure analysis had 12,501 reflections beyond 10.0-Å spacings with $F \geq 3\sigma(F)$. These constitute about 78% of the theoretically observable reflections between 10.0- and 3.1-Å spacings; an additional 5% of the reflections between 3.1 and 3.0 Å are present in this data set.

The structure was determined by molecular replacement (13) using a predecessor of the program package MERLOT (14). Rotation and translation searches were performed independently (15) for the lysozyme, Fv (module containing V_H and V_L, the variable domains of the H and L chains), and C_L/C_H1 (constant domain of L chain/first constant domain of H chain) portions of the structure. In the search for the orientation of the lysozyme and the Fv, the highest peaks in the rotation function turned out to be the correct peaks. The correct peak in the rotation search for the C_L/C_H1 was only the seventh highest. The translation search gave unambiguous results in all three cases. Details of the molecular replacement analysis will be published elsewhere (S.S., E.A.P., G.H.C., and D.R.D.). The molecular probes that proved useful in the analysis were hen egg white lysozyme from the refinement analysis of Diamond (16) [Protein Data Bank (PDB) File 6LYZ], the Fv of McPC603 (17) (PDB File 1MCP), and the C_L/C_H1 of HyHEL-5 (2) (PDB File 2HFL). The orientations and positions of the various parts of the complex were refined with COREL S (18) allowing the V_L, V_H, C_L and C_H1 domains and lysozyme to move independently. The structure was then subjected to restrained least-squares

Abbreviations: H, heavy; L, light; V_L and V_H, variable domains of L and H chains; C_L and C_H1, constant domain of L chain and first constant domain of H chain; Fv, module containing V_L and V_H; CDR, complementarity-determining region; CDR*n*-L or CDR*n*-H, *n*th CDR of L or H chain.

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§Using PEG immunoprecipitation at pH 7.2, Denton and Scheraga determined association constants of 1.5 × 10^9 M^{-1} and 2.5 × 10^9 M^{-1} for HyHEL-10 and HyHEL-5, respectively. Lavoie *et al.* (5) determined association constants of ≈4 × 10^9 M^{-1} and ≈1.4 × 10^{10} M^{-1} at pH 8.2 by the method of Friguet *et al.* (6). The association constant for D1.3 Fab, also determined by the method of Friguet *et al.*, has been reported (7) as 4.5 × 10^7 M^{-1} at pH 7.4; more recently,

refinement using the program PROLSQ (19, 20) and model rebuilding on the basis of OMIT maps (21) using the graphics program FRODO (22). The final R value was 0.24 with deviations from ideality of 0.011 Å for bond lengths and of 0.034

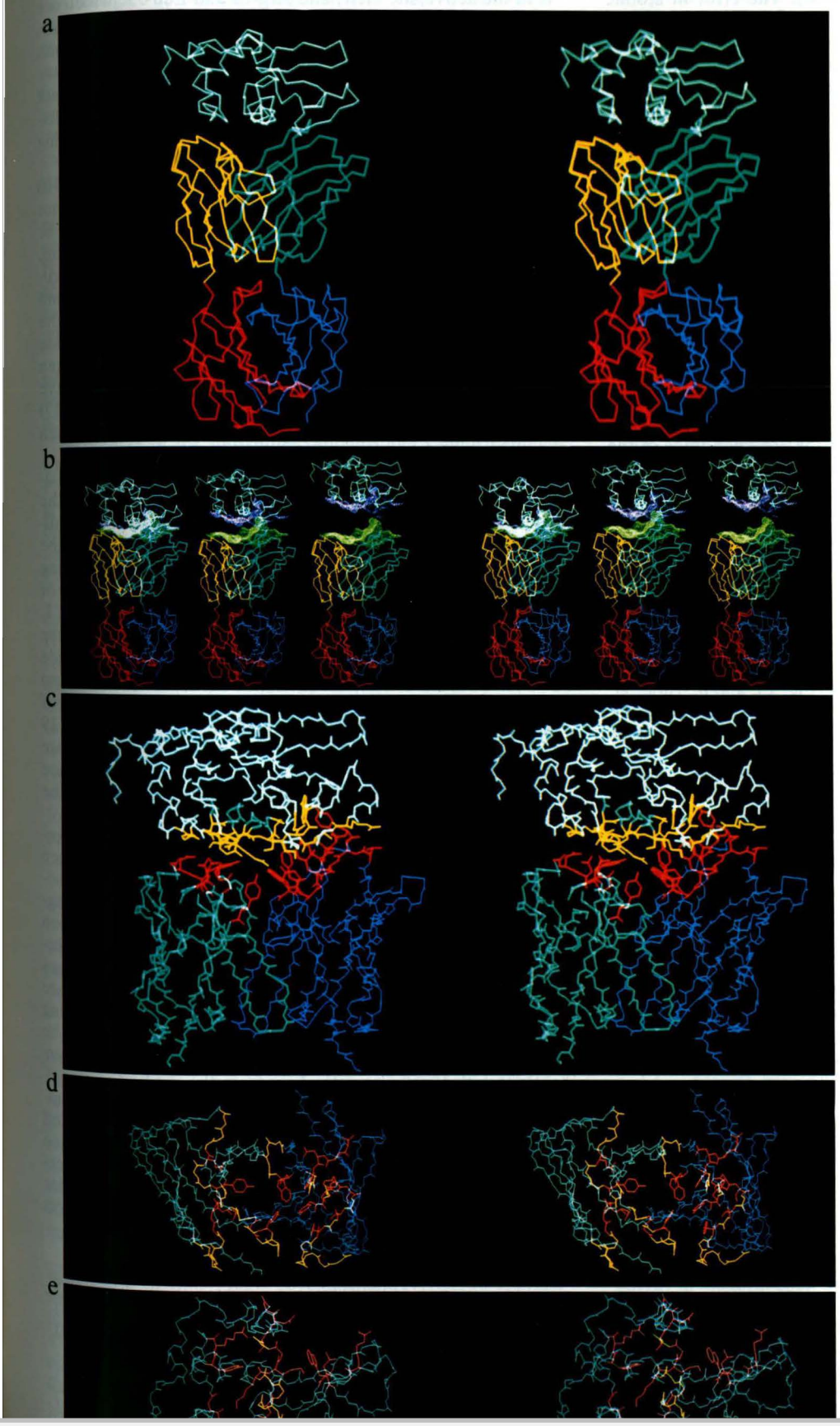

FIG. 1. Stereo diagrams. (*a*) α-Carbon trace of the HyHEL-10 Fab–lysozyme complex. Lysozyme is shown in white, V_L in yellow, V_H in light blue, C_L in red, and C_H1 in dark blue. (*b*) Same as *a* and showing the interacting surfaces: the surface covering the epitope in green and the surface covering the contacting residues from the Fab in magenta. At left, the complex is as it is in the crystal structure; in the middle and at right, the lysozyme has been separated from the Fab by 7 Å and by 14 Å, respectively. (*c*) Backbone of HyHEL-10 Fv and lysozyme with the contacting side chains from HyHEL-10 shown in red and those from the lysozyme shown in yellow. The rest of the helical region (lysozyme residues 88–99) and V_L are shown in light blue, and V_H is shown in dark blue. (*d*) HyHEL-10 Fv showing the CDRs in yellow and the contacting residues in red. V_L is on the left (light blue) and V_H is on the right (dark blue). (*e*) The HyHEL-10 epitope on lysozyme showing the contact-

Å for angle distances and with a deviation from planarity of 0.004 Å. The refined coordinates have been deposited in the Protein Data Bank (23) (File 3HFM). The error in atomic positions was estimated (24) to be 0.4 Å.

Molecular surface representations were computed with the program MS (25) using a probe radius of 1.5 Å and standard van der Waals radii (26). Atomic contacts were defined according to the criteria of Sheriff *et al.* (27). The various domains of HyHEL-10 Fab were compared with the following immunoglobulin structures: McPC603 and J539 (28) (PDB File 1FBJ), HyHEL-5 and D1.3 (courtesy of R. Poljak, Pasteur Institute), KOL (29) (PDB File 1FB4), NEW (30) (PDB File 3FAB), and REI (31) (PDB File 1REI). Least-squares superposition of structures was accomplished with the program ALIGN (written by G.H.C.); only α carbons were used in the superpositions. ALIGN reports the individual deviations and the rms deviation between structurally equivalent pairs of atoms. The numbering scheme used here for the HyHEL-10 residues follows the convention of Kabat *et al.* (32).

RESULTS

Overall Structure. Fig. 1*a* shows the α-carbon trace of the HyHEL-10 Fab–lysozyme complex. The contact between lysozyme and HyHEL-10 involves the complementarity-determining regions (CDRs) of the antibody with the exterior of the lysozyme helix (residues 88–99) and some surrounding amino acid residues. The two interacting surfaces (Fig. 1*b*) are strikingly complementary so that solvent is completely excluded from the interface. The helix in the epitope is oriented diagonally across the combining site so that its N terminus interacts with the second CDR of the L chain (CDR2-L) whereas its C terminus and the segment beyond it interact mainly with CDR1-H and CDR2-H (Fig. 1 *c* and *d*; Table 1).

The Epitope. The lysozyme epitope for HyHEL-10 is quite discontinuous, consisting of residues coming from distant parts of the linear sequence but made contiguous by the folding of the protein. The area of lysozyme that is in contact with the antibody is 774 Å^2.

The lysozyme residues that contact the antibody are His-15, Gly-16, Tyr-20, and Arg-21, which are on one side of the helix; Thr-89, Asn-93, Lys-96, Lys-97, and Ile-98, which constitute the external surface of the helix; Ser-100, Asp-101, and Gly-102, which extend beyond the helix; Trp-63, which is in the active-site cleft; and Arg-73 and Leu-75, which are on the other side of the cleft (Fig. 1*e*). In addition, Asn-19, Asn-103, and Ala-107 are partly buried by the interaction with the antibody, although not in actual contact by the criteria we have used. Four of these residues participate in the contact with the antibody only through their main-chain atoms (His-15, Gly-16, Ile-98, and Gly-102). Most of the contacting residues are polar and five of them are charged.

Table 1. HyHEL-10 residues in contact with lysozyme

HyHEL-10 residue*	Lysozyme residue(s)
V_L	
Gly-30	Gly-16
Asn-31 (h)	His-15, Gly-16, Lys-96
Asn-32 (h)	Gly-16, Tyr-20
Tyr-50	Asn-93, Lys-96
Gln-53 (h)	Thr-89, Asn-93
Ser-91 (m)	Tyr-20
Asn-92 (m,h)	Tyr-20, Arg-21
Tyr-96 (h)	Arg-21
V_H	
Thr-30†	Arg-73
Ser-31 (h)	Arg-73, Leu-75
Asp-32 (s)	Lys-97
Tyr-33 (h)	Trp-63, Lys-97, Ile-98, Ser-100, Asp-101
Tyr-50 (h)	Arg-21, Ser-100
Ser-52 (h)	Asp-101
Tyr-53 (h)	Trp-63, Leu-75, Asp-101
Ser-54	Asp-101
Ser-56	Asp-101, Gly-102
Tyr-58 (h)	Arg-21, Ser-100, Gly-102
Trp-95	Arg-21, Lys-97, Ser-100

*Nature of interaction is indicated in parentheses: m, main-chain

Structure of the Combining Site. The surface of HyHEL-10 that interacts with lysozyme is unusual in that it is not noticeably concave and contains no pronounced grooves or cavities. On the contrary, the surface has a large protrusion, which fits into the active-site cleft of lysozyme. This protrusion is formed by the side chains of Tyr-33 from CDR1-H and Tyr-53 from CDR2-H (Fig. 1*b*). The interacting surface of the antibody contains a disproportionate number of aromatic side chains that point outward and that interact with the antigen (Fig. 1*c*; Table 1). Large numbers of aromatic residues have also been observed in the combining sites of McPC603 (17) and D1.3 (1) and in the presumed binding site of the human class I major histocompatibility antigen A2 (33).

All six CDRs participate in the interaction with the lysozyme. The CDRs of the L chain contribute 8 residues to the contract and those of the H chain contribute 10. One additional residue from the H chain, Thr-30, comes from the framework. CDR2-H has the largest number of contacting residues with 6, while CDR3-H has only 1 (Table 1). For 3 of the residues (Gly-30, Ser-91, and Asn-92, all from the L chain), only their main-chain atoms are involved in the contact. Seven of the contacting residues have aromatic side chains: Tyr-50 and -96 from the L chain; Tyr-33, -50, -53 and -58 and Trp-95 from the H chain. Only one side chain, that of Asp-32 of the H chain, is charged. In addition to the 19 contacting residues, Ser-93 and Trp-94 of the L chain are partly buried by the interaction with the antigen. The surface area on the antibody that is buried by the interaction with the lysozyme is 720 Å^2.

Conformational Changes in the Antigen. No major conformational changes occur in the structure of the lysozyme when it binds to HyHEL-10. Comparison of the complexed lysozyme with the uncomplexed structure (coordinates of tetragonal lysozyme courtesy of D. C. Phillips) gives a rms deviation of 0.47 Å for corresponding α carbons, with significant differences occurring at positions 47, 101, and 102 having deviations of 1.44, 1.80, and 2.13 Å, respectively. Larger differences are found for the side chains, most notably with the aromatic ring of Trp-62, which has been rotated by 150 degrees about the C^β—C^γ bond presumably in order to avoid close steric interactions with a tyrosine side chain from the antibody.

Forces Between the Antibody and the Antigen. The complementarity of the contacting surfaces of HyHEL-10 and lysozyme is so great that there are no cavities in the interface large enough to accommodate a water molecule. The interaction between the two proteins (Fig. 1*c*) consists of polar and apolar interactions; of the 126 pairwise atomic contacts

Table 2. Hydrogen bonds between HyHEL-10 and lysozyme

V_L	Lysozyme	V_H	Lysozyme
Asn-31 OD1	Lys-96 NZ	Thr-30 O	Arg-73 NH1
Asn-32 ND2	Gly-16 O	Ser-31 OG	Arg-73 NH1
Gln-53 OE1	Asn-93 ND2	Tyr-33 OH	Lys-97 O
Gln-53 NE2	Asn-93 OD1	Tyr-50 OH	Arg-21 NH1, Ser-100 O
Ser-91 O	Tyr-20 OH	Tyr-53 O	Asp-101 OD1
Asn-92 O	Arg-21 N	Tyr-58 OH	Gly-102 N

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