NOVEMBER 1987
VOLUME 84
NUMBER 22

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

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

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 issue Number 1, January 1987.

Subscriptions: All correspondence concerning subscriptions should be addressed to the Circulation Office of the PROCEEDINGS. Subscriptions are entered on a calendar year basis only. For 1988, subscription rates are as follows—in the United States: student/postdoctoral, $70; personal, $200; institutional, $250; elsewhere: student/postdoctoral, $110; personal, $240; institutional, $290. Information regarding air mail postage rates is available from the Circulation Office. Subscribers are requested to notify the Circulation Office of the PROCEEDINGS 6 weeks in advance of any change of address; also the local postmaster. The Academy is not responsible for nonreceipt of issues because of an improper address unless a change of address is on file. The notice of address change should list both the old and new addresses. Claims for replacement copies will not be honored more than 60 days after the issue date for domestic subscribers and not more than 90 days after the issue date for foreign subscribers.

Back Issues: Volumes 79–83, January 1982 and thereafter, are available from the Circulation Office of the PROCEEDINGS. The price of a single issue is $13.00.

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.*

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8075–8079, November 1987
Immunology

Three-dimensional structure of an antibody–antigen complex

(immunoglobulins/epitope/x-ray crystallography/complementarity/lysozyme)

STEVEN SHERIFF*, ENID W. SILVERTON*, EDUARDO A. PADLAN*, GERSON H. COHEN*, SANDRA J. SMITH-GILL†, BARRY C. FINZEL‡§, AND DAVID R. DAVIES*

*Laboratory of Molecular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD 20892; †Laboratory of Genetics, National Cancer Institute, Bethesda, MD 20892; and ‡Genex Corporation, Gaithersburg, MD 20877

Contributed by David R. Davies, July 30, 1987

ABSTRACT **We have determined the three-dimensional structure of two crystal forms of an antilysozyme Fab–lysozyme complex by x-ray crystallography. The epitope on lysozyme consists of three sequentially separated subsites, including one long, nearly continuous, site from Gln-41 through Tyr-53 and one from Gly-67 through Pro-70. Antibody residues interacting with lysozyme occur in each of the six complementarity-determining regions and also include one framework residue. Arg-45 and Arg-68 form a ridge on the surface of lysozyme, which binds in a groove on the antibody surface. Otherwise the surface of interaction between the two proteins is relatively flat, although it curls at the edges. The surface of interaction is approximately 26 × 19 Å. No water molecules are found in the interface. The positive charge on the two arginines is complemented by the negative charge of Glu-35 and Glu-50 from the heavy chain of the antibody. The backbone structure of the antigen, lysozyme, is mostly unperturbed, although there are some changes in the epitope region, most notably Pro-70. One side chain not in the epitope, Trp-63, undergoes a rotation of ≈180° about the C^{β}—C^{γ} bond. The Fab elbow bends in the two crystal forms differ by 7°.**

Until recently knowledge of the structural aspects of antibody–antigen interactions has been based on the x-ray analysis of four Fab structures and on some complexes with hapten (1–5). Haptens were observed to bind in grooves or pockets in the combining sites of the New and McPC603 Fabs, and these occupied a small fraction of the total available area of these sites. When haptens bind to these Fabs, no large conformational change occurs. However, one cannot rule out the possibility that the behavior of antibodies would be different when they are bound to larger antigens, such as proteins. For example, the interaction with a much greater fraction of the combining site might in itself be sufficient to induce conformational changes in the antibody. Also, the interacting surfaces might not possess the grooves and pockets observed for haptens, but might resemble more closely the kind of surface observed in other protein–protein interfaces, where exclusion of bound water is believed to play a key role. For this reason we undertook several years ago to investigate the crystal structures of complexes of the Fabs of several monoclonal antibodies to hen egg white lysozyme complexed with the lysozyme (6). In this paper we report the analysis of two different crystal forms of one of these complexes.

The site on the lysozyme to which the antibody binds has been the subject of an extensive serological analysis (7) through a study of cross-reactivity with different avian lysozymes. The results of that analysis are in striking agreement with the crystal structure observations and will be discussed.

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

During the course of this analysis two reports of related x-ray studies of Fab–antigen complexes have appeared (8, 9), one being a description of another lysozyme–antilysozyme complex, although to a different epitope of the lysozyme, and the other describing a complex with the neuraminidase of influenza virus. The observations and conclusions from these two investigations differ in important ways from one another, and we describe below how our results can be related to them.

MATERIALS AND METHODS

Monoclonal antibody (mAb) HyHEL-5 and the Fab–lysozyme complex were prepared as described previously (6, 7). Crystals were grown by vapor diffusion against 20% (wt/vol) polyethylene glycol 3400 (Aldrich) in 0.1 M imidazole hydrochloride, pH 7.0, 10 mM spermine with an initial protein concentration of 7 mg/ml. The crystals grow polymorphically in space group $P2_1$, differing principally in the length of the b axis, which was observed to vary between crystals in an unpredictable manner between 65 Å and 75 Å.

One set of data on a crystal with cell dimensions of a = 54.9 Å, b = 65.2 Å, c = 78.6 Å, and β = 102.4° was collected at Genex (Gaithersburg, MD) using a single detector–single axis Nicolet–Xentronics (Madison, WI) area detector; 20,074 observations yielded 7565 unique reflections. Lorentz, polarization, and absorption corrections were applied (10), and the different frames were scaled together giving an overall merging R_{sym} of 0.044, where $R_{sym} = \Sigma_{hkl}\Sigma_i|\bar{I} - I_i|/\Sigma_{hkl}\bar{I}$. Greater than 90% of the theoretical data were observed to 4.5 Å spacings, greater than 60% from 4.5 Å to 4.0 Å spacings, and about 40% from 4.0 Å to 3.0 Å spacings.

A second data set was collected on a crystal with cell dimensions of a = 54.8 Å, b = 74.8 Å, c = 79.0 Å, and β = 101.8° at the University of California, San Diego, using the Mark II multiwire detector system with two detectors (11); 38,689 reflections were collected from one crystal, of which 15,673 were unique. Lorentz, polarization, and absorption corrections were applied, and the different frames were scaled together giving an overall merging R_{sym} of 0.044. Of the 15,673 unique reflections, 15,166 are within 2.66-Å resolution (86.3% of the theoretically observable); there are an additional 507 reflections between 2.66 and 2.54 Å resolution (18.7% of the theoretically observable).

The structure was determined by molecular replacement using the program package assembled by Fitzgerald (12). Three probes were used: (*i*) tetragonal lysozyme (2LYZ) deposited by R. Diamond in the Protein Data Bank (13); (*ii*) $C_L + C_H1$ of the McPC603 Fab (4); and (*iii*) $V_L + V_H$ of the

Abbreviations: CDR, complementarity-determining region; CDRs 1, 2, and 3 for the light chain are referred to as L1, L2, and L3, and for the heavy chain as H1, H2, and H3; mAb, monoclonal antibody; C, constant region; V, variable region.

McPC603 Fab with the following residues removed from the model: V_L—27C–31 and 91–95; and V_H—30–31, 52B–54, 61–64, 96–100I, and 101. Residue numbering in Fabs throughout this paper follows Kabat *et al.* (14); C and V represent constant and variable regions, respectively, and L and H refer to light and heavy chain, respectively. We oriented McPC603 Fab so that the axis of the elbow was parallel to the z axis, which allowed us to observe most of the difference in elbow bend directly in the γ rotation function angle (15). The fast-rotation function (16) was used with 10- to 4-Å resolution data and a radius of integration of 24 Å. The rotation function of Lattman and Love (17) was used to refine the position of the peak for each probe. The Crowther–Blow (18) translation function was then used with 10- to 4-Å resolution data and a step size of 0.02 unit cell lengths in a and c to determine the x,z translations. We also used the program BRUTE, written by M. Fujinaga and R. Read, University of Alberta, with 5- to 4-Å resolution data and 1-Å step size to determine x,z translations. Where the two methods agreed, we used BRUTE to hold one or two probes stationary and search for the translation of the second or third probe to solve the problem of relative origins in space group $P2_1$. The resulting model from this analysis was examined with the program FRODO (19) on an Evans and Sutherland (Salt Lake City, Utah) PS300 picture system to ensure that the three probes were assembled in a plausible manner and that the crystal contacts were reasonable.

The positions of the Fab and lysozyme were refined using the stereochemically restrained least-squares refinement package PRECOR/CORELS (20). We first refined with three "domains," lysozyme, $V_L + V_H$, and $C_L + C_H1$, starting with 10- to 8-Å resolution data and then extending the refinement to 7-Å spacings and finally to 6-Å spacings. The crystallographic $R = \Sigma_{hkl}||F_o| - |F_c||/\Sigma_{hkl}|F_o|$ for the long b-axis form was 0.44 and for the short b-axis form was 0.49. We then divided the complex into five domains: lysozyme, V_L, V_H, C_L, and C_H1. At this point we replaced the V_L domain of McPC603 with that of antibody J539 (5), because it shares 75% sequence identity with the V_L domain of mAb HyHEL-5 (21). We also replaced the C_H1 domain of McPC603, which is a murine IgA, with the C_H1 domain of antibody KOL (3, 13), which is a human IgG1 and shares 60% sequence identity with the murine IgG1 C_H1 domain of mAb HyHEL-5. At this stage of refinement for both crystal forms the R was 0.42 for 10- to 6-Å resolution data.

At this point we concentrated on the long b-axis form because the data set extended to higher resolution. We removed from the model the side chains of amino acids that were not identical to the mAb HyHEL-5 sequence (ref. 21 and A. B. Hartmann, C. P. Mallett, and S.J.S.-G., unpublished work) and also omitted entire residues of the following complementarity-determining regions (CDRs): L3 (residues 91–95), H2 (residues 52B–54), and H3 (residues 97–100); (see abbreviations footnote). We refined the model against data from 10- to 2.5-Å resolution using the stereochemically restrained least-squares refinement package PROTIN/PROLSQ (22). We first refined only the positional parameters using an overall isotropic B until the $R = 0.344$. We then used FRODO to rebuild the model. In the electron-density map at this stage there was excellent density for the deleted L3 and moderately good density for the deleted H2 and H3. Also, in most cases, electron density was apparent for side chains that had been omitted from the model. Following this, we refined the model adding individual isotropic B factors until the $R = 0.270$, and then we examined the model with molecular graphics and rebuilt parts of the model, especially H3. We once again refined until the $R = 0.249$. At this stage we included an overall anisotropic ΔB (23), which had values of

ing the lattice contacts along the b axis are weak. We then omitted all of the interacting residues in lysozyme, refined the model, calculated an electron-density map, and examined it to determine whether our interpretation of the position of these residues was correct. We did the same thing with the interacting residues on the Fab. After making minor changes to the structure we refined again, yielding $R = 0.245$ with an rms deviation from ideal bond lengths of 0.012 Å. We have deposited the coordinates from this stage of the refinement in the Protein Data Bank (13).

We have estimated the rms positional error to be 0.40 Å by the method of Luzzati (24). In describing the results, hydrogen bonds and salt links were limited to pairs of appropriate atoms with an interatomic distance of <3.4 Å. Maximum van der Waals contact distances were defined as in Sheriff *et al.* (25). Contacting surface area was calculated with program MS (26) using a probe radius of 1.7 Å and standard van der Waals radii (27).

RESULTS

Fig. 1*a* shows the C^α skeleton of the HyHEL-5 Fab–lysozyme complex. V_H and V_L, and C_H1 and C_L adopt the canonical relationships observed in other Fabs. The main difference between the two crystal forms is the elbow bend of the HyHEL-5 Fab, which is 161° in the long b-axis form and 154° in the short b-axis form.

The contact between the antibody-combining site and the lysozyme epitope is extensive and involves many residues. The calculated buried surface (solvent inaccessible) area is about 750 Å^2 on the surface of both the Fab and lysozyme (≈14% of the surface). The interaction between the two proteins is very tight, and there are no water molecules between the combining site and the epitope. The current model contains three salt links and ten hydrogen bonds. Glu-50 (H2) forms salt links to both Arg-45 and Arg-68 of lysozyme, and Glu-35 (H1) forms a salt link to Arg-68. There are 74 van der Waals contacts.

The epitope on lysozyme consists of three oligopeptide segments (Fig. 1*b*). The first consists of Gln-41, Thr-43, Asn-44, Arg-45, Asn-46, Thr-47, Asp-48, Gly-49, and Tyr-53, which are in contact, and Thr-51 and Asp-52, which are partly buried by the interaction. This segment is essentially one long continuous subsite, which consists of two β-strands connected by a bend involving residues 46 through 49 (28). The second segment consists of the contacting residues Gly-67, Arg-68, Thr-69, and Pro-70; and the partly buried residues Asn-65, Asp-66, Gly-71, and Ser-72. This second segment has the form of a rambling loop and contains part of an extensively studied, disulfide-linked antigenic peptide that elicits antibodies that could cross-react with native lysozyme (29). Arg-68 in this subsite has been identified as a "critical" residue to the epitope (7). The third segment consists of the directly interacting Leu-84 and the partly buried residues Pro-79, Ser-81, and Ser-85. Arg-61, which is in none of the segments, is also partly buried upon complex formation. The surface of the epitope is extensive (23 Å between the most distant C^α atoms) and relatively flat except for a protruding ridge made up of the side chains of Arg-45 and Arg-68, and curling back at the edges (Fig. 1*d*).

The antibody-combining site involves residues from all six CDRs (30). Each of these CDRs contributes at least one residue to the interaction with lysozyme, and most contribute several residues (Fig. 1*c*). Trp-47, which is considered part of the heavy chain framework, also interacts with lysozyme. The other interacting residues are Asn-31, Tyr-32, Asp-40, Trp-91, Gly-92, Arg-93, and Pro-95 from the light chain; and Trp-33, Glu-35, Glu-50, Ser-54, Ser-56, Thr-57, Asn-58, Glu-95 and Tyr-97 from the heavy chain. Additional residues

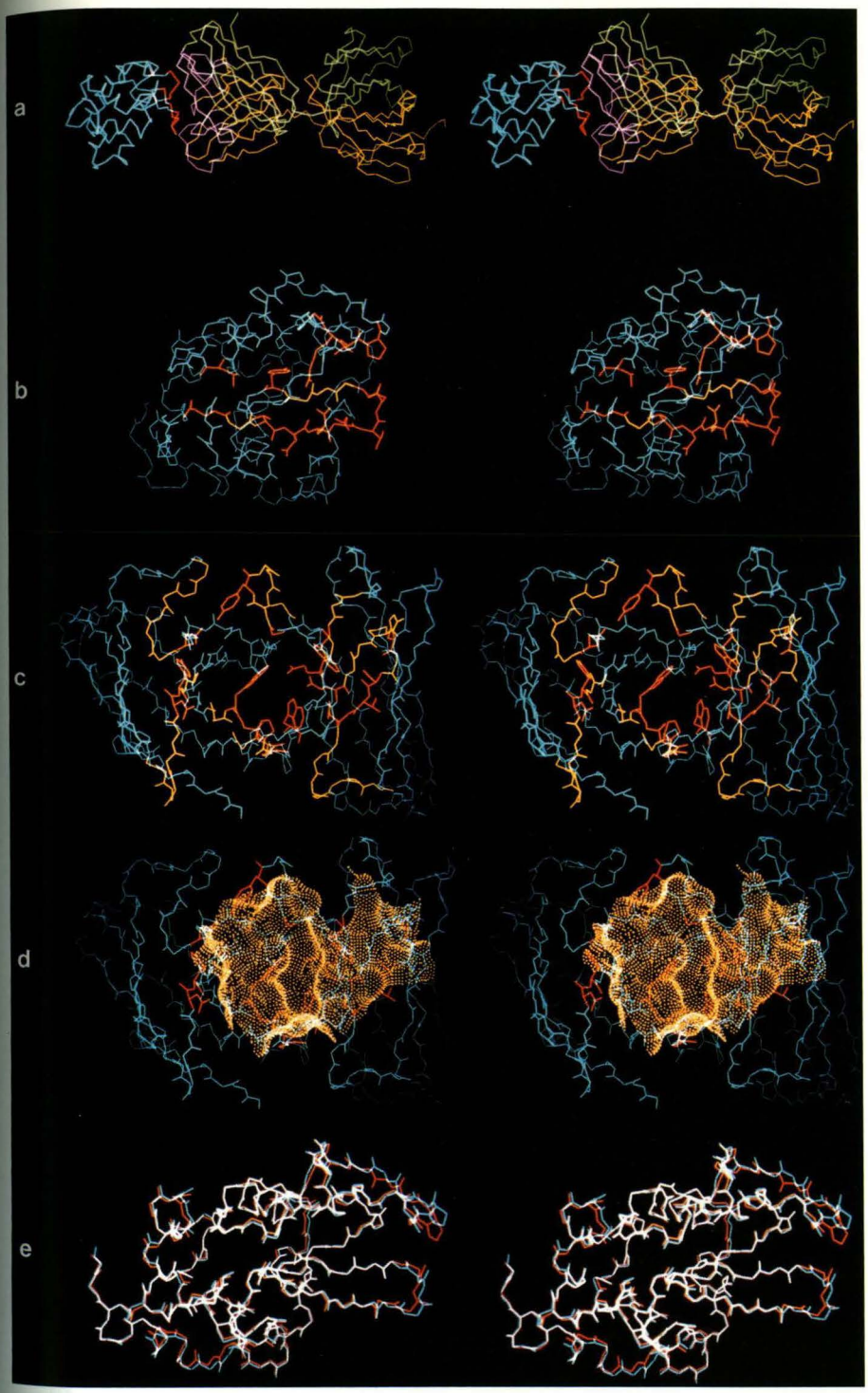

FIG. 1. (*a*) Stereo diagram of C^{α} trace of lysozyme–HyHEL-5 complex. Lysozyme (blue), lysozyme epitope (red), light chain (yellow), heavy chain (green), and CDRs (magenta). (*b*) Stereo diagram of lysozyme highlighting epitope. Residues not in contact with mAb HyHEL-5 have only backbone atoms shown (blue). Residues in epitope (red) and residues in the first subsite not directly in contact with the Fab (yellow). The first subsite (residues 41 through 53) is toward the bottom of the figure, starting at Gln-41 at the lower left and going across to Thr-47 at lower right and then looping back from Asp-48 to Tyr-53; the second subsite (residues 67 through 70) is at the upper right; and the third subsite (Ile-84) is toward the upper left. The side chains of Arg-45 and Arg-68 are more or less vertical and just to the right of center. (*c*) Stereo diagram of mAb HyHEL-5 Fab highlighting CDRs and contacting residues. Framework residues (blue) and CDR, but not in contact (yellow), have only backbone atoms shown. Contacting residues (red) are shown *in toto*. The light chain is on the left, and the heavy chain on the right. L1, lower left; L2, upper left; L3, center bottom; H1, upper right; H2, lower right; and H3, center top. Trp-47 from the heavy chain framework is just to the right of L3, and Glu-35 (H1) and Glu-50 (H2) are directly above Trp-47. (*d*) Stereo diagram of mAb HyHEL-5 with complementary surface of lysozyme epitope superimposed. Residues not in contact with lysozyme (blue). Residues in contact with lysozyme (red). Lysozyme buried surface (yellow dots). Orientation is identical to Fig. 1*c*. Bright areas around edges illustrate curling of surface. (*e*) Stereo diagram of superposition of backbone atoms of lysozyme in tetragonal crystal form on lysozyme in complex with mAb HyHEL-5. Tetragonal lysozyme (blue). Lysozyme in complex with mAb HyHEL-5 (red). White results from exact superposition of red and blue. Epitope is at the right. Pro-70 at upper right can be seen to differ in the two structures. Loop at Thr-47 and Asp-48 also shows differences in backbone at lower right.

framework region 2) from the light chain and Ser-30, Asp-31, Tyr-32, Leu-52, Gly-55, Asn-96, and Asp-98 from the heavy chain. Only 30% of the residues in the CDRs are actually in contact with lysozyme. If one adds the residues that are at least partly buried, this fraction rises to 48%. The surface of interaction is quite broad and extensive with the C^{α} atoms of interacting combining site residues separated by as much as 28 Å. There is a groove on the surface of the antibody running from L3 to H3 and between Trp-91 of L3 and Trp-33 of H1 (Fig. 1*d*). Arg-45 and Arg-68 of lysozyme fit into this groove, placing them in position to form salt links to Glu-50 (H2) and Glu-35 (H1).

association. The structure of hen egg lysozyme has been determined in four crystal forms, thus providing a database for comparing the structure of lysozyme in different environments. The tetragonal lysozyme coordinates used in this comparison (D. C. Phillips, personal communication) differed slightly from those used for structure determination (rms difference = 0.32 Å for 516 main-chain atoms). We superimposed the tetragonal lysozyme coordinates onto the lysozyme in our complex, using all main-chain atoms, both including and excluding atoms from residues that are involved in interactions with the antibody-combining site on the Fab. Qualitatively we see the same behavior with both

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.