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Loss of coactivation function in the SRC-1 null mutants may be partially compensated by increased expression of the closely related coactivator TIF2. Certain clinical syndromes of partial hormone resistance in which receptors are intact might be explained by impairment of nuclear receptor coactivators.

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17. The GLVP cassette encodes a transcriptional regulator composed of the activation domain of VP-16, the DNA binding domain of gal4, and the mutated ligand-binding domain of PR. This regulator can be activated only by a progesterone antagonist, RU-486 [Y. Wang, J. Xu, T. Pierson, B. W. O'Malley, S. Y. Tsai, Gene Ther. 4, 432 (1997)]. This RU486-dependent transrepressor could control a transgene containing a gal4-binding site. The GLVP regulator was incorporated in case the SRC-1 mutation resulted in a lethal phenotype.
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27. We thank K. Bramlett and L. Hadsell for technical assistance; C. Funk, Y. Wang, Z.-Q. Ma, A. Pereira, and N. McKenna for discussion; P. Soriano for the mouse genomic library; A. Bradley for ES cells; D. P. Edwards for the SRC-1 antibody; P. Chambron for the TIF2 cDNA; and J. D. Chen for the RAC8 cDNA. Supported by a NIH NRSA fellowship (J.X.) and NIH grants (B.W.O.).
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Docking Phospholipase A$_2$ on Membranes Using Electrostatic Potential–Modulated Spin Relaxation Magnetic Resonance

Ying Lin, Robert Nielsen, Diana Murray, Wayne L. Hubbell, Colin Mailer, Bruce H. Robinson,* Michael H. Gelb*

A method involving electron paramagnetic resonance spectroscopy of a site-selectively spin-labeled peripheral membrane protein in the presence and absence of membranes and of a water-soluble spin relaxant (chromium oxalate) has been developed to determine how bee venom phospholipase A$_2$ sits on the membrane. Theory based on the Poisson-Boltzmann equation shows that the rate of spin relaxation of a protein-bound nitroxide by a membrane-impermeant spin relaxant depends on the distance (up to tens of angstroms) from the spin probe to the membrane. The measurements define the interfacial binding surface of this secreted phospholipase A$_2$.

Many interfacial enzymes such as phospholipases are water-soluble and must bind to the membrane-water interface in order to hydrolyze components of the membrane. Although the high-resolution structures of aqueous forms of several phospholipases and lipases are known (1), there are no reports that reveal the positioning of an interfacial enzyme at the membrane-water interface. The same can be said for most membrane-bound proteins. In the case of 14-kD secreted phospholipases A$_2$ (sPLA$_2$s), such as bee venom phospholipase A$_2$ (bvPLA$_2$), the interfacial recognition surface is thought to surround the active site slot; the latter is a deep cleft into which a single phospholipid molecule enters to reach the catalytic residues (2) (Fig. 1). Here we describe a high-resolution structure determination tool based on electron paramagnetic resonance (EPR) spectroscopy that allows peripheral membrane proteins such as sPLA$_2$s to be oriented with respect to the membrane-aqueous interface.

EPR methods have been developed that make use of protein site-specific spin labeling and spin relaxants for probing the membrane penetration depth of segments of integral membrane proteins that pass through the membrane (3). In theory developed below, it will be shown that the efficiency of relaxation of a protein-bound nitroxide spin probe by a water-soluble spin relaxant such as tris(oxalato)chromate(III) (Croix) is dependent on the positioning of the membrane with respect to the spin probe, even when the probe is exposed to the aqueous phase. By measuring the Croix-dependent relaxation of several nitroxides placed at defined locations on the surface of bvPLA$_2$, both in the presence and absence of membranes to which the enzyme binds, it is possible to position the enzyme on the membrane.

In order to apply this method to bvPLA$_2$, 13 site-selectively spin-labeled enzymes were prepared (4, 12) with the spin label located on or near the putative interfacial recognition surface (1, 2) and 1 with the probe on the opposite side. The ability of Croix to relax the spin label of each bvPLA$_2$ mutant can be quantified by obtaining the continuous-wave EPR spectra as a function of microwave irradiation power. This series of experiments was carried out in the presence and absence of 10 mM Croix for the enzyme in the aqueous phase or bound to small unilamellar vesicles of the nonhydrolyzable, anionic phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphomethanol (DTPM) (5). bvPLA$_2$ binds tightly to such vesicles (6). For each data set, the power dependence of the peak to peak height of the central line of the first derivative EPR spectrum, $\Delta Y$, was fit by least squares to the power saturation roll-over equation (3, 7)

$$\Delta Y = \frac{c h_1}{(1 + (h_1 P_2)^{-\alpha})^{\varepsilon}}$$

where $h_1 = \sigma P_2^{1/2}$ is the microwave amplitude in gauss, $P_2$ is the power incident on the sample, and $\alpha$ is the conversion efficiency factor for the resonator (5) (4.5 G/W$^{1/2}$). The quantities $c$, $\varepsilon$, and $P_2$, were

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allowed to vary during curve fitting. The parameter \( c \) is a scaling factor, and \( P_0 \) is a power parameter that depends only on the properties of the nitroxide (7):

\[
P_2 = R_2 \cdot R_1, \quad R_1 = \frac{1}{\gamma_1 T_1}, \quad R_2 = \frac{1}{\gamma_2 T_2}
\]  

Here, \( R_1 \) and \( R_2 \) are the spin lattice and spin-spin relaxation rates (in gauss) and are related by the electron gyromagnetic ratio \( \gamma \), to the relaxation times \( T_1 \) and \( T_2 \) as shown (7). The parameter \( \epsilon \) is a measure of the curvature of the power dependence and is \( 3/2 \) for a homogenous line and \( 1/2 \) for a completely inhomogeneous line shape (7). This parameter enables us to obtain very high-quality fits to the data, as it absorbs the effects of inhomogeneous broadening and partially slowed rotational tumbling of the nitroxide.

The change in the fitted value of \( P_1 \) on addition of Crox is taken as a measure of the effect of this metal on relaxation. The presence of Crox increases both \( R_1 \) and \( R_2 \) as follows (8):

\[
R_1 = R_1^0 + \chi \cdot [\text{Crox}]
\]

\[
R_2 = R_2^0 + \chi \cdot [\text{Crox}]
\]

where \( \chi \) is the relativity of Crox (9) and the superscript zero refers to the absence of Crox. The quantity \( \Delta P_2 \) is defined as the difference in \( P_2 \) values in the presence and absence \( (P_2^0) \) of Crox:

\[
\Delta P_2 = P_2 - P_2^0 \approx \chi(R_2^0 + R_2^1) \cdot [\text{Crox}]
\]

Thus, \( \Delta P_2 \) is directly proportional to the concentration of Crox in the vicinity of the spin probe; because \( R_2^0 \gg R_1^0 \), the term that is quadratic in [Crox] is small and neglected (8). \( \Delta P_2 \) is measured in the presence and absence of DTPM vesicles, and these two quantities are used to obtain the exposure factor \( \Phi \) as follows:

\[
\Phi = \frac{(\Delta P_2)_{\text{membrane}}}{(\Delta P_2)_{\text{local}}} = \frac{[\text{Crox}]_{\text{local}}}{[\text{Crox}]_{\text{membrane}}}
\]

The superscript "local" refers to the effective concentration of Crox near the spin label and the presence of the membrane reduces this concentration. The equality on the right side of Eq. 5 follows directly from Eq. 4. The quantity \( 1 - \Phi \) is a measure of the ability of the membrane to shield the protein-bound nitroxide from Crox in the aqueous phase (there is negligible Crox in membranes).

Power saturation rollover curves for the mutant in which isoleucine 2 is replaced with spin-labeled cysteine (12C-sl) and K66C-sl are shown in Fig. 2 along with the fit to Eq. 1. Values of \( \epsilon \) and \( P_2^0 \) for all mutants are listed in (10), and values of \( \Phi \) are listed in Table 1. Ideally, one would expect \( P_2^0 \) to be independent of the presence of the membrane, but it is not (10, 11). K66C-sl has its spin label on the face of bvPLA2 that is opposite the putative interfacial recognition surface, and, as expected, \( \Phi \) for this mutant is close to unity (maximum exposure; Fig. 2 and Table 1). At the other extreme are 12C-sl, K14C-sl, and 178C-sl, which display values of \( \Phi \) close to zero (Fig. 2 and Table 1), and thus the membrane confers nearly complete protection from Crox relaxation on these nitroxides. The other nine mutants display \( \Phi \) values of intermediate magnitude (Table 1).
The key to understanding the data in Table 1 is that the highly negative surface electrostatic potential of DTPM vesicles reduces the concentration of anions in solution near the membrane relative to their bulk concentrations. This results from the Boltzmann equation, which says that the concentration of Crox is a function of the electrostatic potential due to the membrane

$$C_{\text{Crox}}(r) = C_{\text{Crox}}(r = \infty) \exp \left( -\frac{z_{\text{Crox}} F \psi(r)}{RT} \right)$$

Here $C_{\text{Crox}}(r)$ is the molar concentration of Crox at a normal distance $r$ from the membrane; $\psi(r)$ is the electrostatic potential; $z_{\text{Crox}}$ is the charge on Crox; and $F$, $R$, and $T$ have their usual meanings. Poisson’s equation describes the electrostatic potential around any set of charges, and for a planar membrane surface of uniform charge density, the potential depends only on $r$. The final result is the Poisson-Boltzmann equation appropriate for a planar charged membrane (12), which can be written as a first-order differential equation as follows:

$$\frac{\partial \phi(r)}{\partial r} = \left[ \frac{8\pi RT}{\varepsilon} - 9 \cdot 10^{12} \right] \sum C_i \exp(-z_i(\phi(r)F)/RT) - 1 \right]^{1/2}$$

Here $C_i$ is the bulk molar concentration of each electrolyte of change $z_i$ in solution, and $\varepsilon$ is the dielectric of bulk water (a value of 78). Given the experimental value of $\phi(0) = -77 \pm 3$ mV for our system (13), Eq. 7 can be solved numerically to obtain $\phi(r)$, and $C_{\text{Crox}}(r)$ is obtained using Eq. 6.

Theoretical exposure factors $\Phi$ ($r$) can be calculated as

$$C_{\text{Crox}}(r)/C_{\text{Crox}}(\infty)$$

(by analogy to Eq. 5) and compared with experimental $\Phi$ values (Table 1) to obtain the normal distance of each spin label to the membrane. To do this, it was assumed that the x-ray structure determined for bvPLA$_2$ in solution [14] is maintained for the enzyme at the interface and that the membrane that contacts the enzyme is a plane. Marquardt-Levenberg regression analysis (15) was carried out by varying the protein-to-membrane distance and the Euler angles for the rotation of bvPLA$_2$ about its center. Several trials were executed with systematic variation of the initial conditions. In all cases, the analysis converged to a single bvPLA$_2$-membrane orientation. Figure 3 shows the remarkably good fit of experimental $\Phi$ to calculated $\Phi$ (16-18) and Fig. 1 shows the derived structure. The data in Table 1 and (10) also show that values of $\Phi$ are significantly larger when the neutral spin relaxant nickel(ethylendiaminediacetic acid) is used instead of Crox, proving that there is a significant electrostatic component to $\Phi$.

Because the effect of Crox on the EPR parameters was measured for bvPLA$_2$, in solution and bound to membranes, to a first approximation the effect of the electrostatic potential at each nitroxide due to the protein alone is removed from the problem because $\Phi$ is the ratio $(\Delta \Phi)_\text{membrane}/(\Delta \Phi)_\text{membrane}$. Strictly speaking, this is true if the electrostatic potential at each spin label of the protein-membrane complex is equal to the sum of the potentials from the membrane and protein alone. To examine this in more detail, we numerically solved the nonlinear Poisson-Boltzmann equation for bvPLA$_2$ bound to DTPM vesicles as given by Fig. 1 in 50 mM monovalent salt solution and for enzyme and vesicles alone (19). The electrostatic potential of the complex was generally similar to the sum of the potentials due to enzyme and vesicles alone. Very close to the membrane [near spin labels at positions 2 and 14], for which values of $\Phi$ near zero were measured (Table 1), however, the low-dielectric enzyme enhances the negative electrostatic potential of DTPM vesicles by causing the Faraday electric field lines to bend around it and increase in density (20). However, this does not affect the conclusion that these residues are closest to the membrane. Overall, the results suggest that the first-order approach of simply ignoring the nonlinear electrostatic effects is valid.

A clear result of the present study is that bvPLA$_2$ sits on the membrane surface rather than digging into the membrane. This is consistent with monolayer pressure studies showing poor penetration of sPLA$_2$ into an anionic phospholipid monolayer at the air-water interface (21). The opening to the active site slot of bvPLA$_2$ faces the membrane (Fig. 1); however, this opening is not firmly against the membrane. This result is unequivocal as several diagnostic spin labels (at positions 51, 53, 82, 85, and 92) are clearly not as close to the membrane as those at positions 2 and 14 (Table 1). This implies that the alkyl chains of a long-chain phospholipid bound in the active site slot of the enzyme at the interface are partly in contact with the interior of the bilayer, with the hydrophobic walls of the active site slot, and with solvent water [because these experiments were done in the presence of CaCl$_2$, a molecule of DTPM occupies the active site of bvPLA$_2$ at the interface (22)].

The surface of bvPLA$_2$ that contains the opening to the active site slot contains eight cationic residues and only one anionic residue. bvPLA$_2$ and other sPLA$_{2s}$ bind more tightly by orders of magnitude to anionic vesicles than to zwitterionic ones, and it has been hypothesized that these surface cations drive interfacial binding by means of electrostatics. However, our recent study shows that these cationic residues, individually and collectively, are not very important for interfacial binding, because mutating them to glutamates has virtually no effect on the binding of bvPLA$_2$ to anionic vesicles (23). The present study shows that the membrane contact surface of bvPLA$_2$ corresponds to a prominent pitch of hydrophobic residues found on all sPLA$_{2s}$ and that all basic residues except K14 are not in close contact with the membrane [see figure 1 of (23)]. The hydrophobic residues are not deeply inserted into the hydrophobic interior of the bilayer but somehow provide a microinterfacial environment that drives interfacial binding to the “polar” phospholipid headgroups (23).

<table>
<thead>
<tr>
<th>Mutant</th>
<th>$\Phi*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I2C-sil</td>
<td>0.01 ± 0.04</td>
</tr>
<tr>
<td>N13C-sil</td>
<td>0.38 ± 0.03 (0.78 ± 0.04)†</td>
</tr>
<tr>
<td>K14C-sil</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>S15C-sil</td>
<td>0.17 ± 0.02 (0.32 ± 0.03)†</td>
</tr>
<tr>
<td>R23C-sil</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>F24C-sil</td>
<td>0.25 ± 0.13</td>
</tr>
<tr>
<td>T51C-sil</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>T53C-sil</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>K66C-sil</td>
<td>0.85 ± 0.08</td>
</tr>
<tr>
<td>I75C-sil</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>F28C-sil</td>
<td>0.27 ± 0.12</td>
</tr>
<tr>
<td>K85C-sil</td>
<td>0.58 ± 0.13</td>
</tr>
<tr>
<td>D92C-sil</td>
<td>0.44 ± 0.03</td>
</tr>
</tbody>
</table>

*Calculated according to Eq. 5 with the use of the experimental EPR data (10). Numbers in parenthesis were obtained with the use of 10 mM nickel(ethylendiaminediacetic acid) instead of Crox.

![Fig. 3. Regression analysis of the bvPLA$_2$-membrane orientation. The solid line shows calculated values of $\Phi$ as a function of the distance from the spin label to the membrane ($r$), and the circles are the experimental $\Phi$ as a function of the modeled distance from the spin label to the membrane for each residue.](image-url)
The nature of these interactions remains to be understood. It is interesting to note that interfacial binding of cellulases to the "hydrophilic" surface of microcrystalline cellulose is driven by hydrophobic residues, including tryptophans on a cellulose-binding domain (24). Finally, the structure shown in Fig. 1 provides a physical basis for the kinetic data that indicate that the interfacial recognition and catalytic sites are distinct (22).

The docking technique described in this study should be useful for determining the relative position of any macromolecule of virtually any size and of known three-dimensional structure with respect to any surface with known electrostatic properties, as long as there are no gross conformational changes in the structures of the components when they bind to each other. However, useful membrane proximity data should also be obtainable for flexible membrane-bound peptides.

REFERENCES AND NOTES___________________________


4. bpyla mutants (2C, N13C, K14C, S16C, R23C, F24C, T51C, T53C, K66C, I78C, F82C, KSBC, and D92C) were prepared by a polymerase chain reaction (PCR)–based method (a list of PCR primers is available from the authors on request), and proteins were obtained as described (R. R. Anand et al., Biochemistry 35, 4591 (1996); T. Duder et al., Biochim. Biophys. Acts 1165, 201 (1992)). Analysis with Elmans's reagent revealed that the sulfhydryl group of the introduced cysteine was blocked, and electro spray mass spectrometry revealed that this sulfhydryl group was disulfide-linked to free cysteine (from the refolding buffer). These surface-exposed disulfides can be selectively cleaved by treating protein [1 mg/ml in 50 mM tris (ph 8.5)] with 0.5 molar equivalents of dithiothreitol (DTT) from a freshly made stock) for 30 min on ice. A small aliquot of the mixture was submitted to centrifugal gel filtration (DG P-6, Bio-Rad, in 500 mM tris (ph 7.5)) to remove DTT, and the remaining sulfhydryl group content of the protein was estimated with Elmans's reagent. This process was typically repeated two to four times until the sulfhydryl group content reached 0.2 to 0.5 protein equivalents. Protein was immediately treated with spin-labeling reagent (REANAL, Budapest, Scheme 1)

\[ \text{SSO}_4^- \]

\[ \text{N}^+ \]

Scheme 1

1 [equivalent based on moles of sulfhydryl group (DTT + protein) from a stock in CH_3CN]. After 40 min on ice, a small aliquot was submitted to gel filtration as above and the void volume with Elmans's reagent revealed the presence of sulfhydryl group, the protein was treated with an additional 0.5 equivalents of spin-labeling reagent. After a final round of centrifugal gel filtration was dialyzed against 5 mM tris (ph 7.5) at 4°C. The sample was concentrated to approximately 10 mg/ml (optical density 280, A_280 = 1 cm^-1) in a Speed-Vac (Savant) and stored at 4°C for 1 month without loss of enzymatic activity. The specific enzymatic activities of all spin-labeled mutants except I78C-sl are within a factor of 2 of that of wild-type enzyme when assayed in the presence of the spin-label agent [e.g., 1 microgram of Crox as a nonparamagnetic electrolyte]. Saturation recovery and pulse double electron resonance (EL-DO) spectra of labeled spin label were measured in the presence or absence of the spin-label agent [e.g., 4-hydroxy-TMP or 4-aminometh-TMP, both labeled at N, with 13N (gifts from A. Beth, Vanderbilt University) at 51°C. Following the protocol [J. J. Yin and J. Hyde, J. Magn. Reson. 74, 82 (1987)], the Heisenberg exchange rate due to bimolecular collisions of 13N and 15N species was calculated. The ratio of exchange rates for the two relaxing agents of differing charge is equal to the ratio of local concentrations of these agents at the labeled spin label location, and thus the ratio of 13N to 15N may be determined (Y. K. Shin and W. Hubbell, Biophys. J. 61, 1443 (1992); J. Carter and W. Hubbell, Biochemistry 15, 4818 (1976)].


16. Spin labels were modeled into bpyla, as follows. From x-ray crystal structure analysis, spin-labeled lysozyme, the spin label is observed to adopt the refolding buffer). These surface-exposed disulfides can be selectively cleaved by treating protein [1 mg/ml in 50 mM tris (ph 8.5)] with 0.5 molar equivalents of dithiothreitol (DTT) from a freshly made stock) for 30 min on ice. A small aliquot of the mixture was submitted to centrifugal gel filtration (DG P-6, Bio-Rad, in 500 mM tris (ph 7.5)) to remove DTT, and the remaining sulfhydryl group content of the protein was estimated with Elmans's reagent. This process was typically repeated two to four times until the sulfhydryl group content reached 0.2 to 0.5 protein equivalents. Protein was immediately treated with spin-labeling reagent (REANAL, Budapest, Scheme 1)
the membrane is of infinite extent. The Euler angles and protein-membrane distance have errors of less than 1° and 2 Å, respectively, as given by the standard error of propagation for nonlinear least squares algorithms. The data for 178C-sil were not included in the fit because they are clearly anomalous (Table 1). Because this mutant has only 7% of the enzymatic activity of the wild type, EPR data are not useful. All fitting was done with MATLAB (Mathworks, Cambridge, MA).

18. Because the Crox-nitroxide spin exchange happens as fast as Crox diffuses to the nitroxide (9), the possibility that the presence of the membrane slows the rate of spin relaxation by slowing the rate of diffusion-limited Crox-nitroxide encounters was also considered, but the calculation (available from the authors on request) shows that diffusional effects only occur if the membrane is <3 Å from the spin label, and thus diffusional effects are not responsible for the deviation of χ from unity measured when the spin label is tens of angstroms away from the membrane.

19. The electrostatic potentials for bVPLA bound to DTPM vesicles (ΔΦw) as indicated in Fig. 1 and for the protein (ΔΦp) and membrane (ΔΦm) were calculated as previously described (N. Ben-Tal, B. Honig, R. M. Petzsch, G. Denisov, S. McLaughlin, Biophys. J. 71, 561 (1996)). Each leaflet of the membrane bilayer consisted of 360 hexagonally packed DTPM lipids, and it was assumed that Ca2+ and one DTPM were bound in the enzyme’s active site. For each spin label, the potential values in the aqueous phase within 5 Å of the nitroxide nitrogen were used to calculate the average potential difference (ΔΦw = ΔΦp - ΔΦm). The calculated potential differences (residue and average ± SD in millivolts) are: 2, −26 ± 15; 13, −7 ± 5; 14, −16 ± 10; 15, −6 ± 8; 23, −12 ± 6; 24, −9 ± 5; 51, −2 ± 3; 53, 0 ± 1; 66, 3 ± 1; 78, −9 ± 3; 85, −6 ± 2; and 92, −3 ± 1.

23. F. Ghomashchi et al., ibid., in press.
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Immunological Origins of Binding and Catalysis in a Diels-Alderase Antibody

Floyd E. Romesberg,* Ben Spiller,* Peter G. Schultz,† Raymond C. Stevens†

The three-dimensional structure of an antibody (39-A11) that catalyzes a Diels-Alder reaction has been determined. The structure suggests that the antibody catalyzes this pericyclic reaction through a combination of packing and hydrogen-bonding interactions that control the relative geometries of the bound substrates and electronic distribution in the dienophile. A single somatic mutation, serine-91 of the light chain to valine, is largely responsible for the increase in affinity and catalytic activity of the affinity-matured antibody. Structural and functional studies of the germ-line precursor suggest that 39-A11 and related antibodies derive from a family of germ-line genes that have been selected throughout evolution for the ability of the encoded proteins to form a polypeptide combining site. Germ line–encoded antibodies of this type, which can rapidly evolve into high-affinity receptors for a broad range of structures, may help to expand the binding potential associated with the structural diversity of the primary antibody repertoire.

The immune system solves the problem of molecular recognition by generating a large library of structurally distinct antibodies and amplifying those with the requisite binding affinity and specificity in an affinity-based selection. By programming this system with chemical information about a reaction mechanism—for example, the structure of a putative transition state—one can examine the evolution of both binding energy and catalytic function (1). Functional and structural analysis of this process can provide insights into both the molecular basis for the remarkable efficacy of this combinatorial system and the mechanisms by which binding energy can be used to lower the activation energies of reactions (1–5). We now describe one such study of the antibody 39-A11 (6), which catalyzes a Diels-Alder reaction, a widely used and mechanistically well studied reaction in organic chemistry, but one that is rarely found in biological systems. The three-dimensional x-ray crystal structures of the 39-A11 Fab-hapten complex and of the germ-line precursor have been determined, and the immunological origins of this and related antibodies have been characterized.

Antibody 39-A11 was generated to the bicyclo[2.2.2]octene hapten 4, a mimic of the boatlike transition state of the Diels-Alder reaction. This antibody catalyzes the cycloaddition reaction of diene 1 and diene 2 to give the Diels-Alder adduct 3 (Scheme 1) (6). Structurally related haptens have been used to generate other antibodies that catalyze Diels-Alder reactions, suggesting that this is a generally realizable design strategy (7, 8). Antibody 39-A11 was cloned and expressed as a humanized chimeric Fab (9), and the structure of the complex of the recombinant 39-A11 Fab fragment and hapten 4 was determined at 2.4 Å resolution (Fig. 1 and Table 1).

Well-defined density for the hapten was observed in the 1Fo − 2Fc omit map (Fig. 1). The hapten is bound in a cleft ~9 Å wide and ~12 Å deep, with ~194 Å2 of the hapten surface (79% of the total solvent-accessible surface excluding the linker arm) buried within the Fab. There are 89 van der Waals interactions and two hydrogen bonds between the hapten and antibody, with most of these contacting the heavy chain. The bicyclo[2.2.2]octene moiety of hapten 4, which corresponds to the cyclic 4 + 2 π electron system of the transition state, is buried in a hydrophobic pocket, free of solvation. The walls of this cavity consist of the side chains of residues PheH100b [anti chain: Asn215, Trp147, Val191, Pro196, Gly193, Thr150, Ala105, and Arg110] (where H and L represent heavy and light chains of the antibody, respectively). The carbonyl oxygen of the carbamate moiety at the bridgehead position of 4 (the C1 substituent in Scheme 1)