Molecular Forces in Antibody Maturation

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Analysis of x-ray crystal structures has clarified the nature of antibody-antigen interactions, and the conformational basis of specificity and affinity, but does not provide a clear picture of the dynamics of antigen recognition. In particular, we know that primary antibodies can bind a wider variety of ligands than their secondary counterparts—which are tuned for high specificity and affinity. Crystal structures show that in the absence of antigen the secondary antibody adopts a structure preformed for binding, but that the primary antibody does not. Our calculations show that the unligated state of the primary antibody has a well-defined structure, fluctuating no more widely than that of the secondary antibody, and undergoes a discrete structural rearrangement in response to ligand.

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Conformational changes are an integral part of the mechanism of function of many proteins. New experimental and theoretical techniques are providing insight into protein dynamics. They supplement x-ray crystallography which provides very detailed, but static, information. Here we investigate the physics of a set of antibodies [1,2].

The vertebrate immune system has evolved sufficient diversity to bind a wide variety of foreign molecules called antigens. When our bodies first encounter a novel antigen, we mount a primary response, based on the antibodies encoded in our genome. The primary response can recognize the entire organic world—but with relatively low specificity and affinity. This is followed by an internal evolutionary process, exploring the neighborhood of the primary antibody sequences, to tune the response in specificity and affinity. This produces secondary antibodies, which are more effective. The process takes several days, which is why it takes about a week to recover from many infections.

It is of great interest to understand, at both the structural and dynamic level, the nature of the determinants of antibody affinity. This would illuminate the biology of the immune response, and improve the precision by which we can design drugs.

In this Letter, we report a study of a related primary and secondary antibody that bind the hapten (antigen fragment) 5-(para-nitrophenyl phosphonate)-pentanoic acid. Schultz and co-workers [3,4] have solved four related crystal structures (Fig. 1): (i) the unligated primary antibody 48G7 (PDB code 2rcs); (ii) the primary antibody with bound hapten (PDB code 1aj7); (iii) a closely-related secondary antibody, unligated (PDB code 1hkl); (iv) the secondary antibody, with bound hapten (PDB code 1gaf).

The affinity of the secondary antibody for the hapten is 10 000 times greater than that of the primary. However, the binding domains of the primary and secondary antibodies differ in only 9 residues out of 221. Some of the differences occur at sites that are far from the binding site and unlikely to affect affinity. The following interesting observation was made [4]: the two ligated states are similar in structure. They share the conformation required to bind the hapten. The structure of the unligated state of the secondary antibody is also similar, but that of the unligated state of the primary antibody is different. The primary antibody is capable of adopting the ligated conformation, but it does not do so in the absence of the hapten. In contrast, in the unligated state of the secondary antibody the binding site *preforms* the conformation complementary to the hapten. The average displacement between the unligated and ligated state for the primary antibody (0.61 Å) is higher than for the secondary antibody (0.38 Å). The displacements were computed by a superposition of the well-fitting substructures of the antigen-binding domains of the corresponding proteins, followed by calculation of the distance between the corresponding α-carbon atoms of all residues

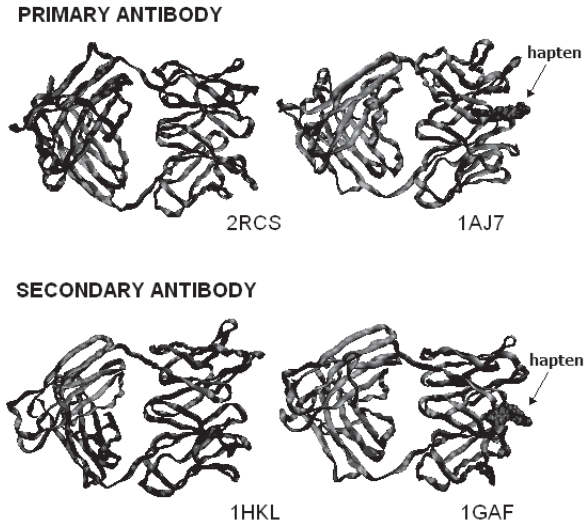

FIG. 1. Structures of primary and secondary antibodies in unligated (left) and ligated (right) states of hapten binding antibody 48G7 [3,4].

(see [5] and references contained therein). Similar results were obtained for antibody and T-cell receptors [6,7].

Because primary antibodies are less specific than secondary antibodies, they are in principle capable of forming different ligated states in the presence of different antigens, as seen with a related system [8]. This raises the following questions, not directly answerable from the static crystal structures: What is the dynamic state of the unligated primary antibody, and how does it recognize and react to encounters with hapten? There are two possibilities: (1) Is the unligated state of the primary antibody in a fixed conformation, (different from the ligated state) and induced to undergo a discrete conformational change to the ligated state when exposed to the hapten? (2) Is the binding site of the unligated state of the primary antibody mobile, including in its ensemble of populated states the conformations that bind? We undertook an analysis of the calculated internal forces and expected flexibility of the structures, to try to answer this question.

The equilibrium dynamics characteristic of proteins can be described by a single-parameter harmonic potential [9–12]. The results from this method (so-called elastic network models) were found to be in excellent agreement with x-ray crystallographic temperature factors (also called Debye-Waller factors) and NMR relaxation data [11].

The elastic network model is based on the following postulate: In folded proteins, residues undergo Gaussianly distributed fluctuations around their mean positions, due to harmonic potentials between all pairs of residues in contact. No residue specificity need be invoked as a first-order approximation. Instead, the inter-residue potentials are all represented by the same single-parameter harmonic potential. The fluctuations of residues are controlled by a harmonic potential and the α carbons are used as representative sites for residues. The dynamic characteristics of the molecule are fully described in this model by the so-called Kirchhoff matrix of contacts. Two residues are defined to be in contact if the distance between their α carbons is less than a cutoff radius of 8 Å. The Kirchhoff matrix, Γ, of contacts and harmonic potential, H, are defined as

$$\Gamma = \begin{cases} -\theta(r_c - r_{ij}) & i \neq j \\ -\sum \Gamma_{ij} & i = j \end{cases}, \qquad (1)$$

$$H = 1/2\gamma(\Delta R^T \Gamma \Delta R),$$

where ΔR is the fluctuation of an α-carbon atom, θ is the step function, and Γ is the Kirchhoff matrix (or the contact map). Note that the generalized inverse of the Kirchhoff matrix is taken here after eliminating the zero eigenvalue. Fluctuations of residues are obtained by inverting the Kirchhoff matrix and given by

$$\langle \Delta R_i \Delta R_j \rangle = 1/\gamma k_B T [\Gamma]^{-1}_{ij}, \qquad (2)$$

where k_B is the Boltzmann constant and T is the absolute temperature.

The x-ray crystallographic temperature factors (also called Debye-Waller or B factors, $B = 8\pi^2\langle \Delta R^2 \rangle/3$) for ligated and unligated states have been experimentally determined by Schultz and co-workers [3,4]. The average fluctuation of the unligated primary antibody (0.74 Å^2) is larger than that of the ligated primary antibody (0.58 Å^2). The results of the elastic network model are in good agreement with these data. The total number of contacts, $\Sigma\Gamma_{ii}$, for the ligated state is larger than that of the unligated state. Therefore, the ligated state should have smaller fluctuations according to the harmonic approximation of the elastic network model [Eq. (2)]. We calculated the average fluctuations at the hapten binding region for unligated and ligated states. The results are plotted in Fig. 2(a) for the four structures. The binding regions of the primary and secondary unligated states are more flexible than ligated states (0.76 Å^2 and 0.64 Å^2, respectively).

We consider a perturbation of the unligated state by an incremental force, Δf, which may displace the coordinates according to the following relationship:

$$\langle \Delta R \rangle_1 = \langle R \rangle_1 - \langle R \rangle_0, \qquad (3)$$

which describes the displacement between the average coordinates of the ligated and the unligated states. Here $\langle \cdot \rangle_1$ denotes the ensemble average of any variable calculated in the ligated configuration as follows:

$$\langle (\cdot) \rangle_1 = \frac{\int (\cdot) Z_1 d\Delta R}{\int Z_1 d\Delta R}, \qquad (4)$$

where the configurational factor for the perturbed state is $Z_1 = \exp\{(-1/2\gamma \Delta R^T \Gamma \Delta R + \Delta R \Delta f_1)/k_B T\}$. It is possible to relate the force, Δf_1, to the conjugate variable ΔR_1. By definition

$$\frac{\partial \ln(\Phi)}{\partial(\Delta f_1)} = \langle \Delta R \rangle_1, \qquad (5)$$

where Φ is the partition function for the ligated configuration defined as $\Phi = \int Z_1 d\Delta R$. We can evaluate the left-hand side of above equation leading to

$$\frac{\partial \ln(\Phi)}{\partial(\Delta f_1)} = \gamma^{-1}\Gamma^{-1}\Delta f_1. \qquad (6)$$

Combining Eqs. (5) and (6), we find the following $\gamma\Gamma\langle \Delta R \rangle_1 = \Delta f_1$. The force is calculated using the unligated state Kirchhoff matrix, Γ; hence the force is the one necessary to get to the conformation of the ligated state.

We calculated the incremental force, Δf_1, which displaces the antibody from the unligated state to ligated state for the primary and secondary antibody. The average force, $\langle \Delta f_1 \rangle$, that is required to move the primary antibody from the unligated state to ligated state is larger (21.4 pN/residue) than the secondary antibody (14.6 pN/residue). The anisotropic network model [11] leads to similar results (61.6 pN/residue and 40.0 pN/residue for the primary and the secondary antibody, respectively). We note that the isotropic model is one dimensional; hence the force values

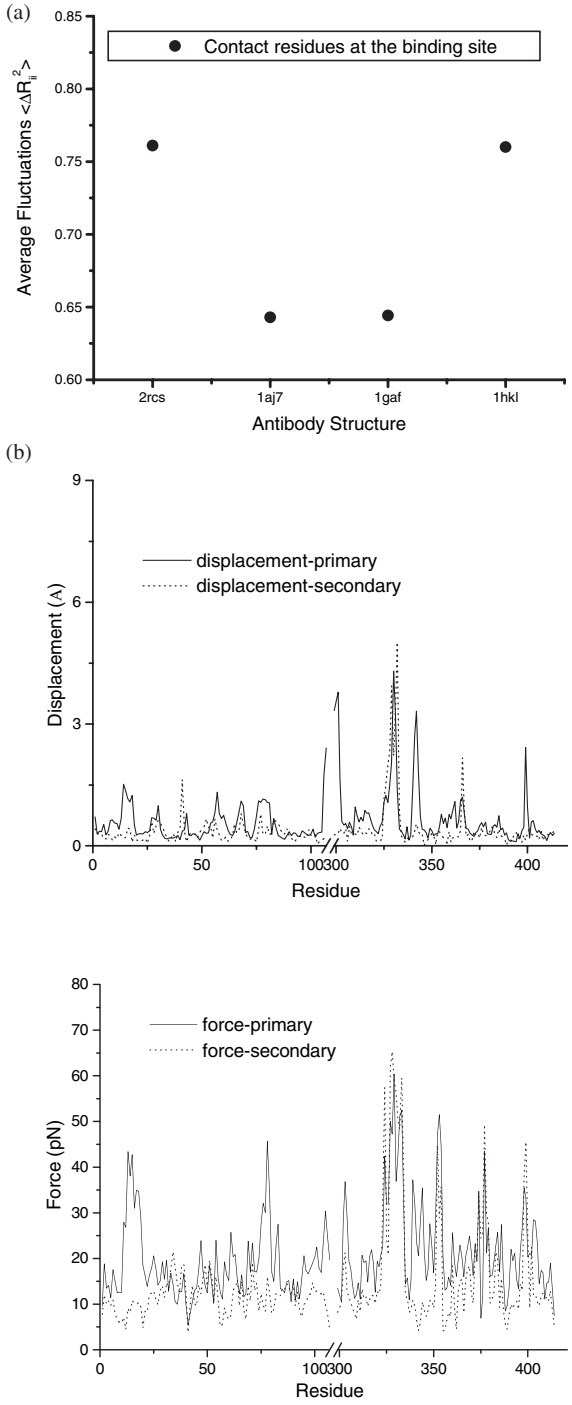

FIG. 2. Average fluctuations for the contact residues at the binding site of hapten (a); force and displacement (b) for V_H and V_L regions of unligated and ligated states of primary and secondary antibody are shown. The incremental force is obtained by $\gamma\Gamma\langle\Delta R\rangle_1 = \Delta f_1$ where $\gamma = 1$ kcal/(mol Å^2) [13].

for the isotropic model are 3 times smaller than the anisotropic network model. Figure 2(b) shows the incremental force and the displacement values for residues 1–107 and 301–414, corresponding to the V_L and V_H domains that contain a binding site. The V_L and V_H domains contain approximately the first hundred residues of the light and heavy chains of an antibody, and include the binding site for antigen. In general, the amplitude of the force is larger for the residues which have large displacement.

These calculations can help choose between the alternative structural hypotheses. If the fluctuations of residues in the binding site of the unligated state of the primary antibody are substantially larger than those in the binding site of the secondary antibody, we would conclude that in the absence of ligand the binding site of the primary antibody is in a mobile dynamic state. If, on the other hand, the magnitudes of the fluctuations are comparable for both states of ligation of both antibodies, we would conclude that the primary antibody undergoes a discrete conformational change between fixed structures upon binding antigen. The latter is observed from our calculations.

In both primary and secondary antibodies, the flexibility of the binding site decreases upon binding to the hapten as a result of protein-ligand forces. However, the flexibility of the binding site of the unligated state of the primary antibody is no more than that of the secondary antibody. This supports the second alternative: we conclude that exposure to hapten induces a conformational change in the primary antibody, from an unligated state that is no less fixed than that of the unligated state of the secondary antibody, to a ligated state of similar structure and flexibility to that of the secondary antibody. Upon encounter with hapten, the primary antibody is exposed to larger forces compared to the secondary antibody. In contrast, binding of the hapten to the secondary antibody is more like a lock-and-key mechanism; the interaction with hapten reduces flexibility but produces substantially less distortion of the structure.

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