J. Mol. Biol. (1998) 278, 301–306

JMB

COMMUNICATION

Ligand-induced Domain Movement in an Antibody Fab: Molecular Dynamics Studies Confirm the Unique Domain Movement Observed Experimentally for Fab NC6.8 upon Complexation and Reveal its Segmental Flexibility

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Two molecular dynamics simulations were carried out for the antibody Fab NC6.8, both with and without the guanidinium sweetener ligand NC174, in order to assess the segmental flexibility as well as the conformational changes upon ligand binding. Trajectory analyses of the simulation of the uncomplexed Fab suggest low-amplitude motions of the Ig domains with respect to each other, most clearly reflected by a periodic alteration of the elbow angle within a range of 11°. Upon insertion of the hapten into the binding site, the quaternary structure of the Fab exhibits considerable rearrangements: the elbow angle changes by almost 30°, the light chain is elongated and the heavy chain becomes more flexed. Comparison with experiment reveals some interesting agreements with X-ray crystallographic results published previously.

Keywords: Fab segmental flexibility; MD simulation; antibody-hapten interactions; conformational changes; anti-sweetener antibody NC6.8

The conformational behaviour of antibodies upon antigen binding is of fundamental interest in structural immunology for the understanding of the humoral immune response. Although a fair amount of X-ray data for antibodies in both their complexed and unbound state is available (Padlan, 1996; Wilson & Stanfield, 1994), a general consensus about antigen binding and signal transmission has not yet emerged, in part due to the lack of detailed dynamical data of the underlying processes (Petsko, 1996). This situation easily leads to controversy when new and unique observations are reported, as in the case of the anti-sweetener antibody NC6.8. The Fab of this antibody has been crystallized and analyzed by X-ray crystallography (Guddat *et al.*, 1994) both with and without the hapten *N*-(*p*-cyanophenyl)-*N'*-(diphenylmethyl)-*N''*-(carboxymethyl)guanidine (NC174) and showed considerable domain rearrangements, reflected most clearly by a large change in the elbow angle (over 30°, Figure 1). These findings gave rise to hypotheses about transmitted conformational changes and intramolecular signalling upon complexation (Guddat *et al.*, 1994, 1995), which, however, were questioned with regard to eventual crystal packing effects, and especially due to the absence of comparable observations for similar systems (Wilson & Stanfield, 1994; Guddat *et al.*, 1995).

In order to shed some light on the matter of dispute, we have carried out molecular dynamics studies for the Fab NC6.8. The primary interest was focused on the differences between the two X-ray structures and the exploration of the possibility to reproduce them by MD simulations in solution, which could, for example, help to validate or disprove the assumption of crystal packing forces as

Abbreviations used: Fab, antigen binding fragment; MD, molecular dynamics; Ig, immunoglobulin; rms, root–mean-square; V_L, V_H, C_L, C_{H1}, variable and constant domains of the light and heavy chain, respectively; NC174, *N*-(*p*-cyanophenyl)-*N'*-(diphenylmethyl)-*N''*-(carboxymethyl)guanidine.

0022–2836/98/170301–06 $25.00/0/mb981684

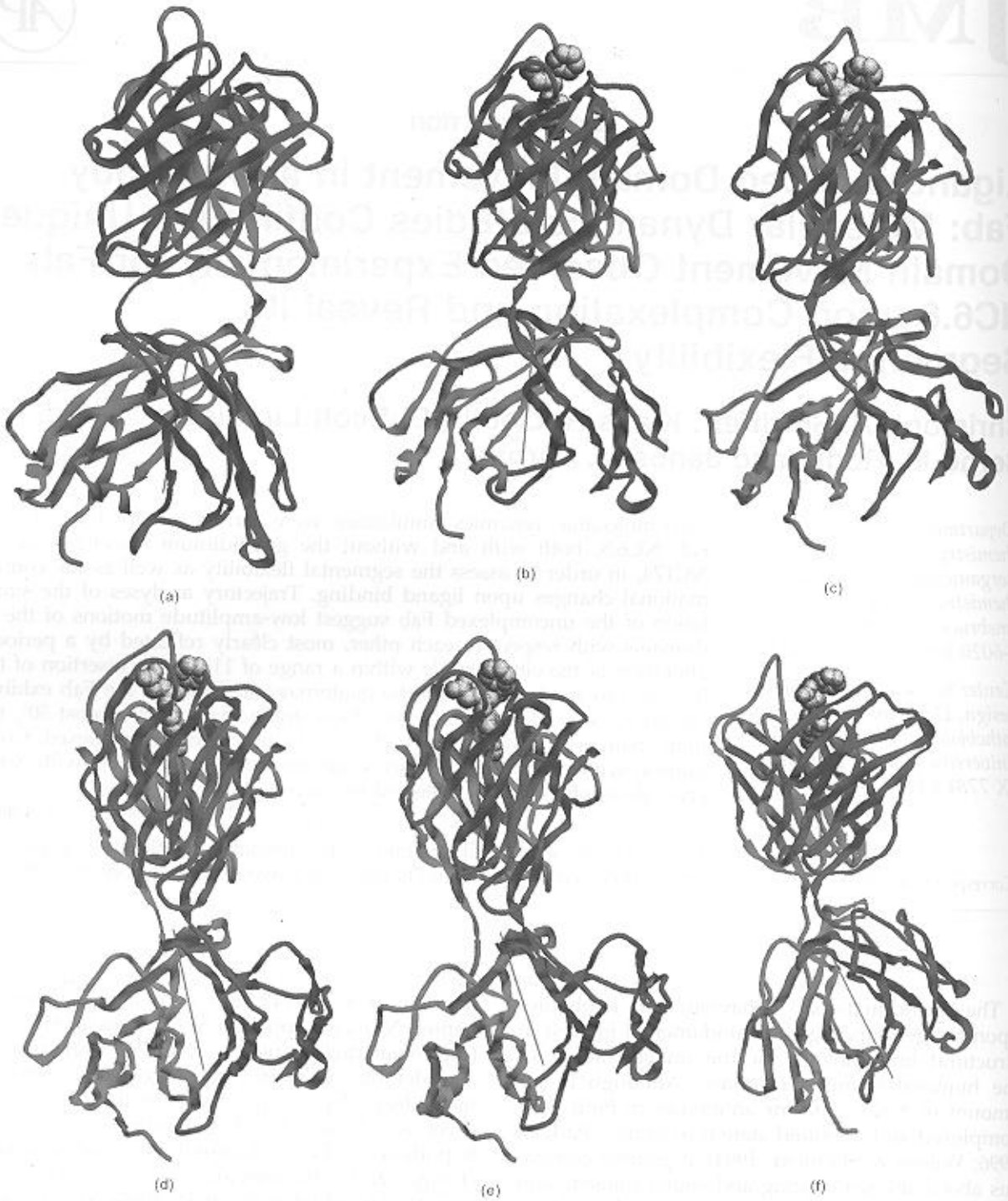

Figure 1. Comparison of the X-ray structure of the unliganded Fab NC6.8 ((a), PDB-entry 1cgs) with snapshots from the MD trajectory of the complexed Fab ((b) 50 ps; (c) 150 ps; (d) 250 ps; (e) 284 ps) and finally with the X-ray structure of the complexed Fab ((f), PDB-entry 2cgr). A Fab consists of four domains, two formed by the heavy chain (H) and two by the light chain (L). The N-terminal structural units are the so-called variable domains V_H and V_L, which together form the antigen binding site; the C-terminal subunits are the constant domains C_{H1} and C_L. Each domain shows the common "immunoglobulin fold" which is a β-barrel of two antiparallel β-pleated sheets. The two domains of the same chain show only very few contacts between each other and are separated by a flexible peptide segment called the switch region. (For detailed recent reviews of antibody structure see e.g. Davies & Chacko, 1993; Padlan, 1994). The Fabs shown here are oriented with the V-domain pair at the top. The axes displayed together with the structure are the pseudodyad axes, defined by rotating the coordinates of the V_L (or C_L) domain into those of the V_H (or C_{H1}) domain. This was done with the help of routines from the ALIGN program (Satow *et al.*, 1986), on the basis of 40 core residues in each of the variable domains and 35 core residues in each of the constant domains. The V-pseudodyad coincides with the z-axis, whereas the C-pseudodyad is oriented parallel with the drawing plane to provide an unambiguous view. In this orientation the pseudodyads give an impression of the elbow angle, which is defined as the angle between the V and C-pseudodyad axis. For the unliganded Fab (a) the elbow angle is 189.3°, for the complexed Fab (f) it is 152.4°. The elbow angles of the snapshots amount to (b) 185.0°, (c) 172.4°, (d) 167.2° and (e) 161.3°.

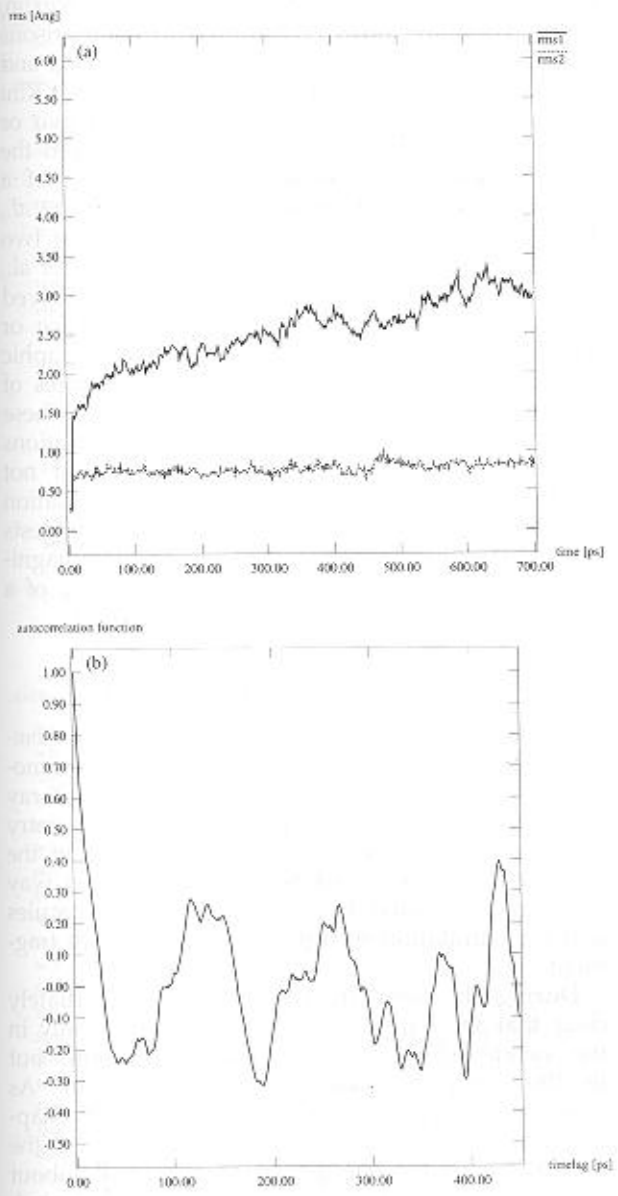

Figure 2. Simulation of the unliganded Fab NC6.8. The MD simulations were carried out with AMBER 4.1 (Pearlman *et al.*, 1994, 1995) using the force-field from Cornell *et al.* (1995). The structure file of the uncomplexed Fab obtained by crystallography (Guddat *et al.*, 1994; PDB entry 1cgs) served as the starting point. Crystallographic water entries were removed and missing hydrogen atoms added, resulting in a total of 6491 atoms for the 433 amino acid residues of the Fab and a net charge of zero. After an initial short minimization (300 steps) to relax internal strains and remove bad contacts, the Fab was placed in a rectangular box of TIP3P water molecules (Jorgensen *et al.*, 1983) such that the minimum solute to wall distance was 8 Å, giving a box size of 94 Å × 75 Å × 62 Å and an amount of approximately 10,500 water molecules. The solvated system was again subjected to minimization (500 steps), mainly to optimize the interactions at the protein-water interface. To equilibrate the system, the protein atoms were first frozen in their position and only the water molecules allowed to move, heated to 300 K over 4 ps and subsequently cooled down again to 80 K in 1 ps. Then the protein atoms were allowed to move as well and the system was heated to 300 K over a period of 15 ps. The simulation was then carried on at 300 K and 1 bar (NPT conditions) up to a total simulation time of 700 ps. Other parameters of the simulation are: full periodic boundary conditions, residue-based dual cutoff for the nonbonded interactions at 8 Å and 10 Å, pairlist update every ten time-steps, time-step of 2 fs, SHAKE (Ryckaert *et al.*, 1977) on bonds to hydrogen atoms, saving energy data every 0.02 ps and Fab coordinates every 0.1 ps. (a) Structural rms deviations from the X-ray structure. rms1 (continuous line) was calculated using all C^{α} atoms of the Fab, while for rms2 (broken line) the C^{α} atoms of the central core of the V_L-domain were used. These were taken from the same 40 residues as in the calculation of the pseudodyad axes. (b) Autocorrelation function of the elbow angle, based on the trajectory from 100 to 700 ps, with a maximum time-lag of 450 ps.

being responsible for the observed structural changes.

The uncomplexed Fab

Starting from the experimentally determined coordinates, the structure was "solvated" by placing it into a box of water molecules and the resulting system was simulated for 700 ps. To our knowledge, this is considerably longer than any other simulation of Fab systems reported so far, which implies greater relevance of statements about dynamical properties. The description of the molecular system was judged to be reliable from an analysis of the time-course of the structural rms deviation of the C^{α} atoms from the starting X-ray structure (Figure 2a). The average value for the last 100 ps is 3.09(±0.11) Å. This is comparable to another Fab simulation (Lim & Herron, 1995), where a main-chain atom deviation of 3.2 Å is reported already after 174 ps. The rms deviation can mostly be attributed to interdomain adjustments and movements, as is obvious from the comparison with the rms deviations calculated for the central core of single domains and shown representatively for the V_L-domain in Figure 2(a) (average over the last 100 ps 0.82(±0.05) Å).

The subsequent analyses were started after the initial 100 ps of simulation to restrict data sampling on the energetically equilibrated part of the trajectory. In order to get an impression of quaternary structure flexibility and dynamics, distance and angular measurements between the so-called Cys-Trp-Cys triads were used, which are highly conserved structural elements present in each Ig β-barrel (triad positions are indicated in the legend to Table 1). The C^{α} atoms of the three residues served to calculate a central triad point, as well as to define a triad plane. The average interdomain distances between these points reproduce the X-ray values very well, as can be seen from Table 1. The only slight deviation is seen for the V_H-C_{H1} distance, which is somewhat shortened compared to the experimental structure. The rms fluctuations of

Table 1. Simulation averages, rms fluctuations and experimental values of various quaternary structural parameters for the unliganded Fab

	Simulation average (100–700 ps)	Rms-fluctuation (100–700 ps)	Experiment (X-ray structure)
A. *C-W-C-triad distances (Å)*			
$V_L - V_H$	22.57	0.55	21.30
$V_L - C_L$	37.28	0.77	37.92
$V_H - C_{H1}$	36.77	0.44	39.71
$C_L - C_{H1}$	21.63	0.62	21.46
B. *C-W-C-triad angles (deg.)*			
$V_L - V_H$	36.22	5.59	37.81
$V_L - C_L$	52.42	6.84	51.80
$V_H - C_{H1}$	62.84	5.72	54.32
$C_L - C_{H1}$	80.98	6.75	70.51
V-pseudodyad	175.2	3.0	178.5
C-pseudodyad	161.5	2.2	167.3
Elbow angle	187.8	2.0	189.3

The Cys-Trp-Cys triad distances were measured between the triad centres defined by the three C^{α} atoms; the corresponding angles were measured between the planes defined by these three atoms. The triads of each domain are formed by the following residues (numbered according to Kabat *et al.*, 1991): V_L Cys L23-Trp L35-Cys L88; V_H Cys H22-Trp H36-Cys H92; C_L: Cys L134-Trp L148-Cys L194; C_{H1} Cys H142-Trp H157-Cys H208. Concerning the pseudodyad and elbow angles, see the legend to Figure 1

the distances range between 0.44 and 0.77 Å, suggesting only low amplitude motions of the domains with respect to each other. Essentially the same picture arises from the angular measurements, where rms fluctuations between 5.6° and 6.8° are observed. Again, the averages reproduce the experimental values, with minor differences in the case of the V_H-C_{H1} angle (corresponding to a slightly greater flexion of the H-chain) and the C_L-C_{H1} domain pair.

Parameters commonly used for the description of the Fab domain orientation are the pseudodyad angles and the elbow angle (Figure 1 and Table 1). The averages of these angles are in good agreement with the experimental values as well and show rms fluctuations of 2 to 3°; the maximum value observed is 194.2°, the minimum value 183.4°. In accordance with the deviation for the C_{H1}-C_L-triad angle, the C-pseudodyad angle is shifted to a lower value in the simulation. The time-course of the elbow angle shows a regular opening and closing, which could be an indication of a periodic hinge-bending motion. In order to investigate this further, the autocorrelation function of the elbow angle was calculated and is shown in Figure 2(b). Characteristic low-frequency periodicities are immediately visible: as revealed by Fourier transformation, a vibration corresponding to a period of 137 ps (0.24 cm^{-1}) prolongs throughout the whole range and is superimposed by a fluctuation of considerably smaller amplitude characterized by a period of 23 ps (1.46 cm^{-1}).

Information about the elbow bend flexibility in Fab fragments has been obtained so far mainly by crystallographic studies (as reviewed for example by Padlan, 1996; Wilson & Stanfield, 1994; Nezlin, 1990). They are, however, limited to comparisons of static images, be it between the unliganded and the complexed state (e.g. Stanfield *et al.*, 1993; Rini *et al.*, 1992), between Fabs crystallized in two or more forms (e.g. Sheriff *et al.*, 1987), between the Fabs present in the same asymmetric unit of a single crystal (e.g. Prasad *et al.*, 1988; Rini *et al.*, 1993) or, as described recently, between the two Fab arms of an intact IgG molecule (Harris *et al.*, 1997). In all these cases, the differences observed are due to different external forces (presence or absence of a ligand, different crystallographic environment) and thus provide only estimates of the intrinsic flexibility of a Fab in solution. These experimentally verified conformational variations in general correspond to elbow changes of not more than 15°. The complementary information obtained by the simulation presented here suggests now that conformational changes of this magnitude are fully compatible with the flexibility of a Fab fragment in solution.

The Fab complexed with hapten NC174

Starting from the X-ray structure of the *uncomplexed* Fab fragment, the hapten was accommodated in the binding site according to the X-ray data available for the complexed form (PDB entry 2cgs). This was done without any change in the binding site. The structure obtained in this way was again solvated in a box of water molecules and the simulation set up as for the free Fab fragment.

During the simulation it became immediately clear that the hapten induces changes not only in the neighbourhood of the binding region, but to the complete structure of the protein. As expected, the perturbation introduced by the hapten caused the system to considerably extend the time required for energetical equilibration (about 200 ps). Already during the equilibration period, interdomain rearrangements took place. These changes are illustrated in Figure 1 as a comparison of characteristic snapshots from the first half of the trajectory. As can easily be seen from the pictorial representation, hapten binding induces changes in the Fab domain orientation very similar to the changes observed by the comparative X-ray study (Guddat *et al.* 1994, 1995).

More quantitatively, the simulation (200 to 700 ps) shows an average for the elbow angle of 168.9° (with a minimum of 160.7°) and variations similar to those observed for the uncomplexed Fab, though with somewhat less pronounced periodicity. The average is closer to the experimental value of 152.4° for the complex than to the starting point of 189.3°. This transition seen for the elbow angle can also be monitored by following the distance between residues located at exposed parts of the V and C domains and separated only by the gap of the switch region, as has been done for a representative set of residues in Figure 3(a). The

quaternary structural changes reflected by the elbow angle also show up in other distance measurements, and all reflect the fact that the L-chain is elongated, while the H-chain becomes more flexed. The V_L-C_L Cys-Trp-Cys-triad distance averaged over the last 200 ps is 40.9 Å (experimental values; complexed Fab 41.3 Å, free Fab 37.9 Å), while the analogous value for the V_H-C_{H1} distance is 36.7 Å (experimental values; complexed Fab 34.9 Å, free Fab 39.7 Å). The end-to-end distances of the L and H-chain shown in Figure 3(b) represent even more clearly the fact that upon ligation significant alterations take place that make the system approach the experimental structure of the *complex*.

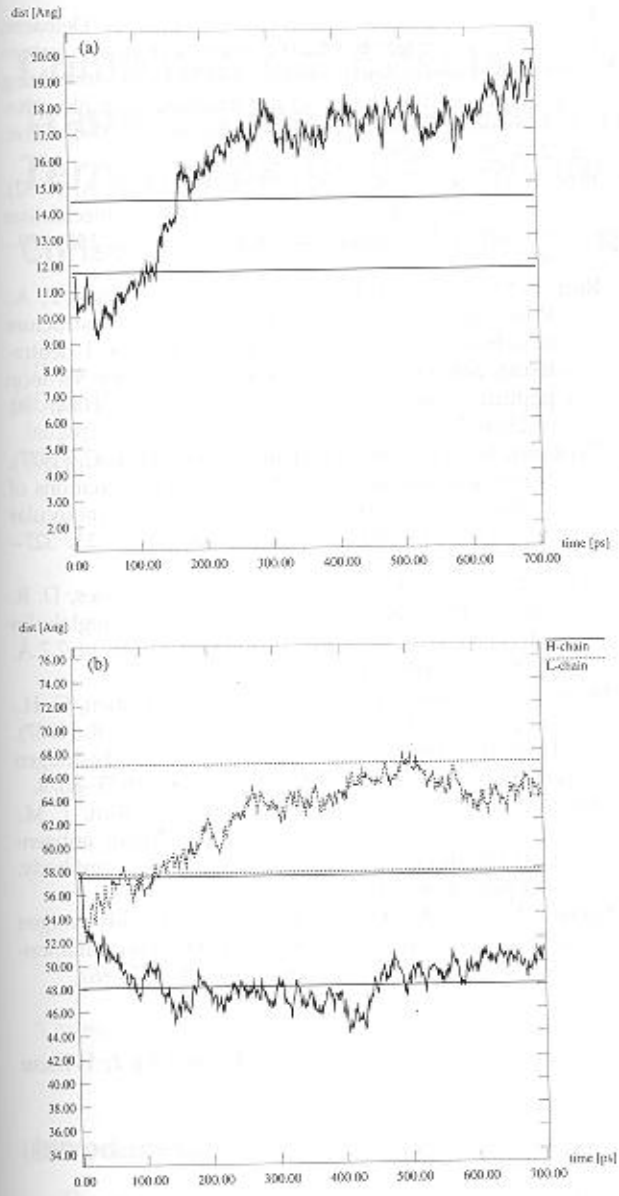

Figure 3. Simulation of the complexed Fab NC6.8 starting from the unliganded structure. The simulation was carried out exactly as described for the unliganded Fab (Figure 2), with the additional presence of the hapten as the only difference. (a) Variation of the distance between the V_L and the C_L domain in the switch-near region, measured between the centres of the backbone atoms of Ser L10–Leu L11 (V_L) and Lys L142–Asp L143 (C_L), residue numbering according to Kabat *et al.* (1991). The straight lines correspond to the values measured for the X-ray structure of the unliganded Fab (11.7 Å) and the complexed Fab (14.5 Å). (b) Variation of the end-to-end distances of the H-chain (continuous line) and the L-chain (broken line), measured between the C^α atoms of the N-terminal and the C-terminal residues of the H and the L-chain, respectively. The two straight lines in the centre correspond to the experimental values of the free Fab (H 57.5 Å; L 57.7 Å), while the lower and the upper straight lines correspond to the experimental values of the complexed Fab (H 67.0 Å; L 48.1 Å).

It is remarkable that the simple insertion of a small hapten into the binding site causes the entire Fab to proceed to a significantly altered quaternary structure in a simulation on the near-nanosecond time-scale. Furthermore, it is intriguing that the alterations clearly exceed the movements revealed by the trajectory of the uncomplexed Fab, and especially that many of the observed changes lead to structural parameters close to the values shown in the experimental complex structure. This demonstrates that the hapten, indeed, is able to elicit large-scale allosteric-like effects that go far beyond localized changes of the induced fit type. To assign a detailed biological significance in terms of signal transduction to these domain movements would be premature. However, the domain rearrangements obtained by simulation prove that the experimentally observed changes in the elbow angle upon complexation cannot simply be attributed, or limited to crystal packing effects.

To increase further our knowledge about the NC6.8 system, additional simulations of the complexed Fab (starting from the X-ray structure of the complex) and of the uncomplexed Fab (obtained by removing the hapten from the X-ray structure of the complex) have been started as well. The results will be presented elsewhere.

Acknowledgements

We thank G. Cohen at the NIH and S. Sheriff at Bristol-Myers Squibb Pharmaceutical Research Institute for providing their program ALIGN and helpful comments on it.

This work was supported in part by grants No. P10229-MED from the Austrian Science Fund to J.M.V. and NIH/NIGMS 46535 to D.S.L.

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Edited by I. Wilson

(Received 11 August 1997; received in revised form 20 January 1998; accepted 22 January 1998)