Somatic mutations in immunoglobulin V gene determine the structure and function of the protein − an evidence from homology modeling

Junko Shimura1, Satoru Miyazaki1, Sachiko Hirose2, Toshikazu Shirai2 and Hideaki Sugawara1,
1 Life Science Research Information Section, The Institute of Physical and Chemical Research (RIKEN)
2−1 Hirosawa Wako Saitama 351−01 Japan
2 Department of Pathology, Juntendo University School of Medicine,Hongo,Tokyo 113 Japan

Correspondent :Junko Shimura, Ph.D. Life Science Research Information Section,
The Institute of Physical and Chemical Research (RIKEN)
2−1 Hirosawa Wako Saitama 351−01 Japan

Abbreviations: CDR complementarity determining region
PCR polymerase chain reaction
FACS fluorescent activated cell sorter

2 Figures 1 Table are attached

ABSTRACT
The antigen binding site of IgG immunoglobulins consists of two separate peptide domains,VH and VL. This part of combined domains contains three loops in each and it provides unique structure to distinguish antigens. The unique sequences encode the antigen binding site are determined by recombination of V region genes in both VH and VL during the differentiation of B−lymphocyte and successive somatic mutations. To investigate the correlation between the site of somatic hypermutations in immunoglobulin V region genes and the affinity maturation of antibodies, single clone derived anti−double stranded DNA antibody−producing hybridomas showing different DNA−binding activities were selected from an autoimmune disease−prone, (NZB x NZW)F1 female mouse. DNA sequence analyses showed that mutational points in V region genes were distributed in both complementality determining regions (CDRs) and framework regions and that there were no common mutational positions that account for the affinity maturation of the clones. The number of cationic amino acid residues did not simply explain the difference in the affinity among these clones too. Studies using homology modeling on a graphic computer showed that changes in the tertiary structure of Fv were minimal between the low and high affinity clones. The

molecular dynamics simulation of the antigen-binding sites, however, revealed a significant increase in the flexibility of antigen-binding loops in high affinity clones. There was a case in that a single mutation in CDR1 of light chain from aspartic acid to glycine markedly increased the vibration of heavy chain CDR3, in association with the increase in DNA-binding activity. The interaction between VH CDR3 and VL CDR1 was only able to be found on the three dimensional graphics by estimating the distance between two separate peptide chains over the period of molcular dynamics simulation. Together with findings by others as well as our own, it is likely that the affinity maturation of anti-DNA antibodies is mediated by two molecular mechanisms caused by somatic hypermutations in V region genes, one by amino acid substitutions to electrophilic residues along the surface of antigen-binding CDR loops particularly of heavy chain CDR3, and one by their increased flexibility particularly between light chain CDR1 and heavy chain CDR3. This explanation is consistent with the induced-fit hypothesis of antigen-antibody interaction. Thus a honology modeling is useful tool for the analysis of the effect of mutaitions in sequences to the function of large bio-molecules.

INTRODUCTION

The unique sequence in immunoglobulin V genes encodes specific antigen binding site of antibody. V gene sequences in heavy and light chains become distinct by recombination of germline V region genes during the differentiation of B-lymphocyte and successive somatic mutations(1-8). The affinity of immunoglobulin has known to be increased during further maturation of B-lymphocyte, that is a consequence of somatic mutations. This is so called affinity maturation. To investigate the the clitical site of somatic hypermutations for affinity maturation, single clone derived anti-double stranded DNA antibody-producing hybridomas were subjected. Because matured B-lymphocytes that derived from single clone are using identical set of VH and VL genes, the definit substitutions of amino acid to influence affinity can be demonstrated by compareing these set of clones. The previous study of three dimensional models of antigen binding site demonstrated whether a cationic residue can locate in the antigen binding site or rather existing outside of the groove structere. Also the result of molecular dynamics simulation suggested that the flexibility of heavy chain CDR3 loop and light chain CDR1 loop are influenced on the affinty changes of such clones' products(9). We attempted explisit water models to investigate further physiological vibration of the loops in the present study.

Materials and Methods

Hybridomas: NZB and NZW strains of mice, originally obtained from Japan SLC Inc.(Shizuoka, Japan), were mated to produce (NZB x NZW)F1 hybrid mice. Spleen cells from a 6 month-old female mouse were fused to the non-secreting myeloma cell line P3-X63-Ag8.653, as described(10). Culture supernatants with binding activities to DNA were screened using ELISA(11). Positive clones were subsequently subcloned at least twice by limitting dilution.
DNA Sequencing of V genes were performed by PCR with method described in (12). DNA binding activity of monoclonal antibodies were determined by FACS using DNA coupled beads and expressed as peak fluorescent intensity as chunnel number(12).
Model building and molecular dynamics simulation: The computer graphic models of VH and VL chains of anti-dsDNA mAbs were built,using Insight II and Homology software

packages(Version 2.3.0, Biosym Technologies, March 1993, San Diego CA USA), on a Silicon Graphics (Indigo2) computer system. The general strategy for the model building was as follows: a) selecting a reference molecule, b) loop searching and loop replacement, when insertion is required, c) checking kirality and d) energy minimization(13). As a reference graphic model for the building of VH and VL, the coordinates of the reported mAb BV04−01, which is reactive with single stranded DNA(14), was used, because of its high sequence homology to V region genes of our clonally related anti−dsDNA mAbs. After refinement by 1000 energy minimization steps, the potential energy of all models was reduced to less than −8000 kcal/mol.

Predicted immunoglobulin molecule with 5 Å layers of bulk water are prepared as the model system for dynamics simulations. Bulk water was added automatically using SOAK command of Insight II program. Molecular dynamics was performed for 50 ps. We took the target tempreture as 298 K, 1 fs time step and 10 Å cut−off distance for non−bonded pair interactions. A package program DISCOVER (Version 2.3.0, Biosym Technologies, March 1993, San Diego CA USA) that bundled generalized valence force field for proteins and nucleic acid dynamics (CVFF).

RESULTS

The mutations found in CDRs were listed in table 1 with annotation of DNA binding activity. The substitutions of amino acid by somatic mutations were distributed in hevy chain CDR1, CDR2, CDR3 and light chain CDR1.The cationic residues that have been considered to be responsible to DNA binding(16) were appeared in heavy chain CDR2 in both lowest DNA binding activity showing clone BW9−7 and intermideate DNA bindig clone BW9−9, but not in BW9−11 that is the highest DNA binding clone. The clone BW9−11 had two arginine residues in tandem in the heavy chain CDR3 but it lacked lysine the other two clones had. The tandem arginine residues of the highest activity antibody (BW9−11) appeared to provide potential electrostatic atomosphare, at leaset,in the area around heavy chain CDR3 in the three dimensional image. The three dimensional models could give a clear view on distribution of cationic residues surroundig antigen binding site (Fig 1).

The characteristic structure of light chain CDR1 was commonly observed in these three monoclonal antibodies. The insersion of 3 additional residues in CDR1 according to Kabat's residue numbering system(17). This has not been observed in most of the immunoglobulin Fab structure stored in PDB, except the anti−nucleotidyl antibody (14). The extended loop structure of light chain CDR1 resulted in the compact groove space that is, the light chain CDR1 loop and heavy chain CDR3 loop locate close. Although predicted model structure convey important information on distinctive narrow groove structure of three anti−DNA monoclonal antibodies, the difference of DNA binding activity was not comprihensively explained with static coordinate data. The trajectories of molecular dyanmics simulation in the presence of water moleclules showed that the antibody of higher DNA binding activity (BW9−9) had more flexible feature than the lower activity antibody (BW9−7) (Fig. 2). Note that the difference of the RMS over the last 30 ps of simulation was significant lthogh it was not as marked as the result in vacuum state in the previous study(9).The decreasing profile of total energy of the models during the simulation appeared to be very similar in both BW9−9 and BW9−7(Fig.2).These observations obtained with monoclonal antibodies with molecular dynamics are valuable to characterize the feature of proteins that includes various biological recognition molecules.

DISCUSSION

The homology modeling with the visualization of flexibility of molecules based on molecular dynamics gave rise to the novel view of analyzing the characteristics of monoclonal antibodies.

The steady state structural analysis of antigen−antibody complex had been performed by x−ray crystallography and static lock−and−key hypothesis had prevailed (18). However, reports that support such dynamic interaction between antigen and antibody as induced−fit increase these days. Wilson et al. (20) have analyzed the shape of the antgen−binding site from a number of Fab complexes with antigens ranging from small haptens to proteins. Although the total surface areas buried by the various antigens is often similar, the surface contours seen by the antigen differ. The difference in the two conformational forms of the Fab (liganded and unliganded) illustrated the induced fit of an antibody to an antigen(19−24). The difference in antigen−binding activity of anti−DNA mAb clones in the previous study (9) was concluded that it was due to the difference in their flexibility of antigen−binding loops. The value of RMS here to determine the structural variance over the simulation was not as large as the result in the absence of water molecules in the previous study (9), it was confirmed that the significant difference of flexibility difference between low binding activity antibody and high activity antibody occur even in the physiological condition.

Thus simulation targeted at the observation of flexibility is a valuable application of molecular dynamics to the characterization of the elucidation of affinty change of monoclonal antibodies induced by somatic point mutaions.

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FIGURE LEGENDS

Fig. 1
The energy minimized structure of monoclonal anti−DNA antibodies, BW9−7, BW9−9 and BW9−11. The top views of antigen binding site are shown. CDR loops are shown with blue ribbon and framework regions are in trace of backbone structure in magenta.The side chains of amino

acid locating in CDR loops were displayed. Yellow side chains indicate that the positions contain cationic residues, lysine and arginine.

Fig.2
The cluster graphs of trajectory of moleclular dynamics simulation. Each pair of the coordinates at every 500 fs in the period of last 30 ps of simulations were superimposed and RMS values were shown in colored dots: RMS 0.00−1.00 were indicated in black, RMS 1.00−2.00 were indicated in yellow, respectively. At the beginning of the simulation the structure was not settled. To avoid the vibration at the high energy state and to observe the vibration in more physiological state, the trajectories of last 30 ps out of 50 ps were compared between BW9−7(above left) and BW9−9(above right). The transition of total energy of each structure were shown at the bottom.

Table 1
Seqeunces in CDRs and DNA binding activities of the monoclonal antibodies derived from single precursor B−lymphocyte

Antibody	Heavy chain CDR sequences		
	CDR1	CDR2	CDR3
BW9−7	SYVIH	YITPYNGGTKYKEKFKG	RRVGKPAWFAY
BW9−9	SYVIH	YITPYNGGTKCKEKFKG	RRVGKPAWFAY
BW9−11	SYVMH	YINPYNGGTKYNEKFKG	RRRGNPAWFAY

	Light chain CDR sequences			DNA binding activity *
	CDR1	CDR2	CDR3	
BW9−7	RASKSVSTSDYNYIH	LASNLKS	QHSRELPYT	192
BW9−9	RASESVSTSGYSYIH	LASNLKS	QHSRELPYT	486
BW9−11	RASKSVSTSGYSYIH	LASNLKS	QHSRELPYT	577

* The fluorescent intensity of DNA−coupled silica gel particles was measured using FACStar(Becton Dickinson, MountainView. CA) in logalithmic amplification. The channel number showing the peak fluorecence intensity on the FACS profile was regarded as a value of the binding activity of anti−dsDNA mAb.