Protein-protein interactions on the surface of immunoglobulin molecules

Wataru Ito,* Haruki Nakamura,† and Yoji Arata*

Department of Biophysics and Biochemistry, Faculty of Science* and Department of Applied Physics, Faculty of Engineering,† University of Tokyo, Tokyo, Japan

Electrostatic potential profiles are visualized for protein-protein interactions on the surface of immunoglobulin molecules. The pH dependence of potential profiles for the facing surfaces of the complex of staphylococcal protein A and the Fc region of IgG was calculated on the basis of the pKa data determined using ^1H NMR spectroscopy for individual His residues. A remarkable electrostatic complementarity between protein A and Fc was observed at high pH, where the stable protein A-Fc complex exists. At low pH, this kind of complementarity is overwhelmed by the repulsion between the maxima of positive potentials that are formed by protonation of the imidazole ring of the His residues. Electrostatic potential profiles were also calculated for the antigen-binding sites of antibody molecules. It was shown that an interesting pattern of the combination of polar and nonpolar regions exists in common in the antibody molecules examined. We suggest that the pattern observed plays a crucial role for transcribing the information of the antigen-binding site.

Keywords: n.m.r.; immunoglobulin; protein A; protein-protein interaction

NOTE: The following abbreviations are used in this article:

Fab	antigen-binding fragment
Fc	fragment composed of the C-terminal halves of the heavy chains
F_B	the B domain of protein A
HEL	hen egg white lysozyme
HPLC	high-performance liquid chromatography
NMR	nuclear magnetic resonance

Address reprint requests to Dr. Arata, at his present address: Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo 113, Japan.

Received 21 June 1988; revised 20 September 1988

INTRODUCTION

Elucidation of the nature of protein-protein interactions, in which multiple characteristics of the molecular surface play a crucial role, is of primary importance in understanding the molecular mechanism of recognition in biological systems. It has been estimated for protein-protein interactions in the insulin dimer, the trypsin-pancreatic trypsin inhibitor complex and the oxyhemoglobin dimer that most of the binding energy derives from hydrophobic interactions.[1] However, this contribution is nonspecific, possibly leading to all kinds of incorrect interactions. We definitely have to have a mechanism with which specificity is produced. It was suggested that the proper formation of hydrogen bonds and of van der Waals contacts, which contribute little to the stability of the complex, would give the complementarity of the surfaces, resulting in the specificity of interactions between two proteins.[1] On the basis of the results of extensive immunochemical studies of the antigenicity of proteins, it has been demonstrated that antigen-antibody interactions are predominantly polar in nature, and stabilizing effects are contributed by hydroxy and nonpolar amino acids through hydrogen bonding and hydrophobic interactions.[2]

Color-coded computer graphics representations of the electrostatic potentials have been shown to be powerful in revealing intermolecular specificity.[3] Nakamura *et al.* have developed another approach, in which (1) the molecular surface is approximated as an assembly of polygons forming a polyhedron and (2) each polygon is color-coded according to the value of electrostatic potential at the center of it.[4] This approach is particularly suited for illustrating the whole tertiary image of the potential distribution on the interacting molecular surfaces.

Staphylococcal protein A is known to bind with high specificity to the Fc region of immunoglobulins. It has been demonstrated that the interaction between protein A and Fc is pH dependent and the pH dependence is different for different classes and subclasses of immunoglobulins.[5] X-ray crystallographic studies have shown that protein A interacts with the junction of the C_H2 and C_H3 domains of Fc.[6]

Electrostatic potential profiles of the molecular surfaces were calculated in the pH range 5–7 for Fc and F_B, which is the B domain of protein A. The pKa values of the imidazole ring of the histidine residues that are involved in the interaction were determined using ^1H NMR spectroscopy. These results will be discussed in terms of the known pH dependence of protein A–Fc interaction.

An X-ray crystallographic study has recently been reported of the complex of hen egg white lysozyme (HEL) and Fab of a mouse monoclonal antibody that was raised against HEL.[7] Electrostatic potential profiles were calculated for the antigenic determinant site on the HEL molecule. The results will be compared with those calculated for four structurally homologous lysozymes obtained from Bobwhite quail, Japanese quail, California quail and turkey. On the basis of the results obtained, we will discuss a possible role of the electrostatic complementarity of recognition in protein-protein interactions. We will also discuss the implications of the present results in terms of the occurrence of "internal images" in idiotype-antiidiotype interactions.[8]

MATERIALS AND METHODS

Protein A was purchased from Nakarai Chemicals in Kyoto, Japan. F_B fragment of protein A was obtained by essentially following the procedure described by Hjelm, Hjelm and Sjoquist.[9] Digestion products were subject to high-performance liquid chromatography (HPLC) on a Mitsui Toatsu HCA-100S hydroxyapatite column. The fraction containing the F_B fragment was further purified on a YMC 312 reverse-phase HPLC column.

^1H NMR spectra were measured on a Bruker WM-400 spectrometer operating at 400 MHz in the Fourier transform mode. NMR measurements were made at 30°C. The pH titration data were obtained in the presence of 0.2M NaCl in D_2O.

A computer program, TERAS, developed by Nakamura *et al.*[4,10] was employed with some modifications for the calculation of electrostatic potential profiles for the interacting surface of protein A and the Fc region of human IgG1. Here, the interacting surface is generated as follows: For each atom of protein A, the nearest atom of Fc is selected and the length L is calculated by subtracting the sum of the van der Waals radii of both atoms from the distance between them. Each one of the atoms of protein A is allocated with a sphere with a radius of the sum of L/2 and the van der Waals radius. A surface is generated by collecting nonoverlapping parts of the surfaces of all the spheres. The interacting surface is then defined as the region of the above surface that faces Fc. Atomic charges of both protein A and Fc are used to calculate individually the potential values on the interacting surface. The electrostatic potentials on the solvent-accessible surface of lysozymes were obtained by averaging the potential for each atomic surface and visualized using color-coded display by computer programs AVEMS and GRAIP.[11] Atomic partial charges were set following TERAS, where it is assumed that Asp, Glu, Lys and Arg residues along with the N- and C-terminal residues are all ionized. For each His residue, an apparent partial charge at pH 7 was calculated by extrapolation using the pK value that was determined by NMR measurements. In order to take into account the effect of shielding by the solvent, a distance-dependent dielectric constant $\varepsilon = r$ was used, where r is the inter-particle distance for any given geometry.[12,13] The atomic coordinates of protein A, Fc of human IgG1 and HEL were obtained from the Brookhaven Protein Data Bank.[14] The coordinates of lysozymes of Bobwhite quail, California quail, Japanese quail and turkey, which are not available at present, were generated from the energy minimized HEL structure by procedures of the minimization of the conformational energy using a program named FEDER.[15]

Table 1. The pKa values of His residues of F_B and Fc*

Fc		
	His-268	6.5
	His-285	7.0
	His-310	7.0
	His-429	7.2
	His-433	7.1
	His-435	6.8
F_B		
	His-137	7.0

*Determined by ^1H NMR measurements[16,17]

RESULTS AND DISCUSSIONS

Fragment F_B of protein A contains one histidine residue at position 137. Plots of the C2-H and C4-H proton chemical shifts as a function of pH for the histidine residue of F_B gave the pKa value, which is included in Table 1 along with those obtained for Fc of human IgG1.[16,17] It was also confirmed by ^1H NMR that the pKa values for F_B and Fc change very little on formation of the F_B-Fc complex.

The electrostatic potential profiles at pH 7.0 are reproduced in Color Plate 1(a), where contributions from protein A and Fc are projected on the interacting surface for the complex. Clearly electrostatic complementarity exists between the positive and negative portions for the two potential surfaces originating from protein A and Fc. Figure 1 also illustrates how the profiles of the potential surfaces change with pH. It was assumed that only the histidine residues are responsible for the pH dependence of the charge of the side chains. Observation of ^1H NMR spectra clearly indicates that no major change in conformation occurs in the pH range 5–7 for the F_B and Fc fragments. As Figure 1 shows, the positive maxima of the potentials originating from the opposite surfaces of the two interacting molecules appear at low pH in the same region (at the low-center part). This would presumably contribute to the repulsive force for the two interacting molecules. This is consistent with

Table 2. Comparison of amino acid sequences of five lysozymes[18]

Lysozyme	Residue 3	15	19[a]	21[a]	40	41	55	68	73	91	99	101	102	103	121[a]
Chicken (HEL)	F	H	N	R	T	Q	I	R	R	S	V	D	G	N	Q
Bobwhite quail	—[b]	—	—	—	S	—	V	K	—	T	—	—	—	—	—
California quail	—	—	—	—	S	—	V	—	—	T	—	—	—	—	H
Japanese quail	T	—	K	Q	—	—	—	—	—	—	—	—	V	H	N
Turkey	Y	L	—	—	—	H	—	—	K	—	A	G	—	—	H

(a) Residues that exist in the *epitope* recognized by the monoclonal antibody under consideration
(b) The residue at the position, where a — is given, is the same as HEL

the fact that the F_B-Fc complex has a tendency to dissociate with a decrease in pH.

On the basis of the results obtained above for the protein A-Fc binding, we will discuss antigen-antibody interactions in which multiple characteristics of the molecular surface play a crucial role. Amit *et al.* have recently published an X-ray crystallographic study of the complex of HEL and Fab of a mouse monoclonal antibody raised against HEL.[7] Of particular interest is the finding that the antibody molecule covers a larger area of the surface of the antigen than previously thought.

The antibody molecule recognizes an immense variety of antigens with the architecture of the antigen binding site fixed, which contains several hypervariable loops. In the case of small hapten molecules, binding energy is derived presumably from a limited number of contacts with the antibody surface. By contrast, protein antigens are fundamentally different from the small hapten molecules in that much larger areas of the antibody surface contact with proteins.[7,19] In spite of this apparent difference in the mode of binding, there is no basic difference in binding energy for these two cases. Close contacts using larger areas of surfaces would have resulted in a much stronger binding than usually observed. These results suggest that the antigen-binding site has been designed in such a way as to express a very broad specificity at a sacrifice of binding energy, coping with an immense variety of antigens.

Amit *et al.* examined binding affinity for four kinds of lysozymes that are highly homologous to HEL.[7] Bobwhite quail lysozyme strongly binds to the anti-HEL antibody, presumably recognizing the same *epitope*, whereas three other kinds of lysozymes from California quail, Japanese quail and turkey do not bind to the antibody at all. Table 2 compares amino acid sequences for all these lysozymes. An electrostatic potential profile was calculated for the antigen-binding surface of HEL and compared with those calculated for the homologous lysozymes from Bobwhite quail, California quail, Japanese quail and turkey (Color Plate 2). Bobwhite quail lysozyme, which does not have any amino acid substitution for the surfaces recognizing the antibody, gives the pattern that is identical with that for HEL. By contrast, lysozymes from Japanese quail and turkey give patterns that are entirely different from that for HEL. The pattern that California quail lysozyme shows is also significantly different, especially in the central part, from that for HEL.

It has been demonstrated that most of the binding energy derives from hydrophobic interactions, but this does not contribute much to specificity.[1] An important characteristic of electrostatic interactions is that they are long-range in nature. Therefore, we suggest that electrostatic potential profiles play an important role at the initial stage of recognition for the two interacting molecules. After the initial encounter, fine-tuning for the closer contact of the two interacting surfaces would obviously be needed for the optimum binding. Hydrophobic interactions may play the dominant role for the stabilization of the complex. Formation of hydrogen bonds and van der Waals contacts would also be crucial for the tight binding, as pointed out by Amit *et al.*[7]

The present result is of interest in view of the occurrence of "internal images" of the original antigen in idiotype-anti-idiotype interactions.[8] For the occurrence of "internal images," we have to have a mechanism with which larger areas of antigen-binding region are transcribed with high fidelity. As discussed above, we cannot expect rigorous topological complementarity; two surfaces facing with each other have charges and groups that can form hydrogen bonds. However, complementarity is poor in these types of interactions. In other words, complementarity would be *dissipated* during transcription of the information of the antigen-binding region. We suggest that the type of complementarity of electrostatic potential profiles as discussed in this paper plays an important role in the occurrence of "internal images" in idiotype-anti-idiotype interactions. With this model, any area of a variable domain, that happens to be sufficiently complementary to an epitope

or idiotype displayed by another molecule can serve as the combining site, or paratope.[20]

REFERENCES

1 Chothia, C. and Janin, J. *Nature* 1975, **256,** 705–708
2 Atassi, M. Z. *Immunochemistry* 1975, **12**, 423–438
3 Weiner, P K., Langridge, J. M., Blaney, J. M., Schaefer, R. and Kollman, P. A. *Proc. Natl. Acad. Sci. USA* 1982, **79,** 3754–3758
4 Nakamura, H., Kusunoki, M. and Yasuoka, N. *J. Mol. Graphics.* 1984, **2**, 14–17
5 Langone, J. J. *Adv. Immunol.* 1982, **32,** 157–252
6 Deisenhofer, J. *Biochemistry* 1981, **20,** 2361–2370
7 Amit, A. G., Mariuzza, R. A., Phillips, S. E. V. and Poljak, R. J. *Science*, 1986, **233**, 747, 753
8 Jerne, N. K. *Ann. Immunol.* (Paris) 1974, **125C,** 373–389
9 Hjelm, H., Hjelm, K. and Sjoquist, J. *FEBS Lett.* 1972, **28,** 73–76
10 Nakamura, H., Komatsu, K., Nakagawa, S. and Umeyama, H. *J. Mol. Graphics.* 1985, **3**, 2–11
11 Nakamura, H., Yamazaki, T., Abe, H., Noguchi, T., Seno, Y., Wako, H. and Go, N. *J. Mol. Graphics.* 1986, **4**, 180–181
12 Warshel, A. and Levitt, M. *J. Mol. Biol.* 1976, **103,** 227–249
13 Gelin, B. and Karplus, M. *Biochem.* 1979, **18,** 1256–1268
14 Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, Jr., E. F., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. and Tasumi, M. *J. Mol. Biol.* 1977, **112,** 535–542
15 Wako, H. and Go, N. *J. Comput. Chem.* 1987, **8**, 625–635
16 Endo, S. and Arata, Y. *Biochem.* 1985, **24,** 1561–1568
17 Shimizu, A., Honzawa, M., Ito, S., Miyazaki, T., Matsumoto, H., Nakamura, H., Michaelsen, T. E. and Arata, Y. *Molec. Immunol.* 1985, **20,** 141–148
18 Smith-Gill, S. J., Wilson, A. C., Potter, M., Prager, E. M., Feldmann, R. J. and Mainhart, C. R. *J. Immunol.* 1982, **128,** 314–322
19 Berzofsky, J. A. *Science* 1985, **229,** 932–940
20 Jerne, N. K., Roland, J. and Cazenave, P.-A. *EMBO J.* 1985, **1,** 243–247