Dendritic Antibody Supramolecules: Combination of IgM and IgG

Akira Harada,* Hiroyasu Yamaguchi, Kaori Tsubouchi, and Eri Horita
Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043

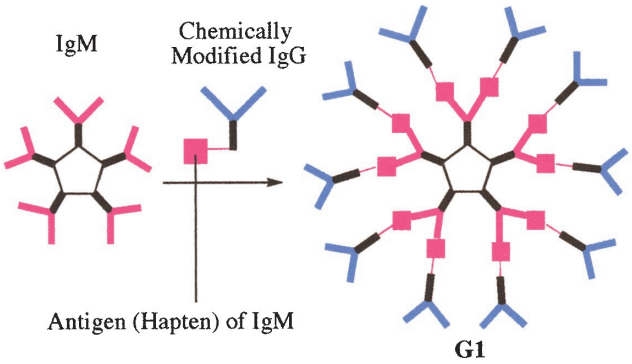
(Received September 6, 2002; CL-020767)

A novel antibody supramolecule was designed and prepared by using immunoglobulin M (IgM) as a core and chemically modified IgGs as branches. The dendritic supramolecules were composed by proteins with molecular weight of about 2 million and constructed by non-covalent bonds. They could bind antigens strongly with high specificity.

Recently, much attention has been directed toward antibodies not only in the field of biology but also in the field of chemistry because of their unique structures and functions. Antibodies, immunoglobulins, have been studied as sensors,[1,2] diagnostics,[3,4] DDS,[5,6] catalysts (catalytic antibodies),[7–9] and components for nano-technology.[10] There are some kinds of antibodies. Immunoglobulin G (IgG)[11,12] is a basic type of antibody, consisting of two heavy peptide chains with molecular weight of about 50000 and two light chains with molecular weight of about 25000. There are two identical binding sites at the top of Fab fragments of IgG which are bound by flexible hinges with a single constant stem (Fc). IgG takes Y or T-shape. IgG is generated in a final stage of immunization, so it is matured and highly selective. IgM has a pentameric structure of IgG and ten antigen binding sites in a single molecule.[13] It binds large multivalent antigens strongly (avidity) because of their multivalent structures. However, IgM is generated in an initial stage of immunization, so it is unmatured and less specific for the antigen than IgG. In order to design an antibody system with a high specificity and a high affinity, a combination of the functions of both IgG and IgM seems to be important. Previously, we reported preparation and properties of IgG oligomers.[14] Now we designed and prepared dendritic antibody supramolecules, in which IgM[15] was placed in a core and many IgGs were bound around the IgM.

A monoclonal antibody (IgM) for cationic porphyrin has been prepared using [5-(4-carboxyphenyl)-10,15,20-tris(4-methylpyridyl)]porphine (3MPy1C) as a hapten. IgG specific for anionic porphyrin, *meso*-tetrakis(4-carboxyphenyl)porphine (TCPP), has been prepared.[16–18] The cationic porphyrin, 3MPy1C has been attached to the IgG via activation of carboxylic acid in 3MPy1C using the condensation agent, carbonyldiimidazole. The IgG-cationic porphyrin conjugate was purified by a column chromatography using Sephadex G-150 to remove the porphyrins that did not react to the antibody. The characteristic binding ability and specificity of IgG were found to remain during the chemical modification of IgG with 3MPy1C. Scheme 1 shows the route for the construction of the dendritic supramolecules. When IgM for 3MPy1C is treated with IgG covalently bound cationic porphyrin, IgM binds the cationic porphyrin attached on the IgG to give a dendritic antibody supramolecule "antibody dendrimer" (**G1** in Scheme 1).

The binding property of the antibody dendrimer (**G1**) with a cationic- or anionic-porphyrin was measured by the enzyme-linked immunosorbent assay (ELISA). Figure 1(a) shows the



Scheme 1. The synthetic route of the complete antibody dendrimer. An ideal structure of the dendritic supramolecule is shown as **G1**.

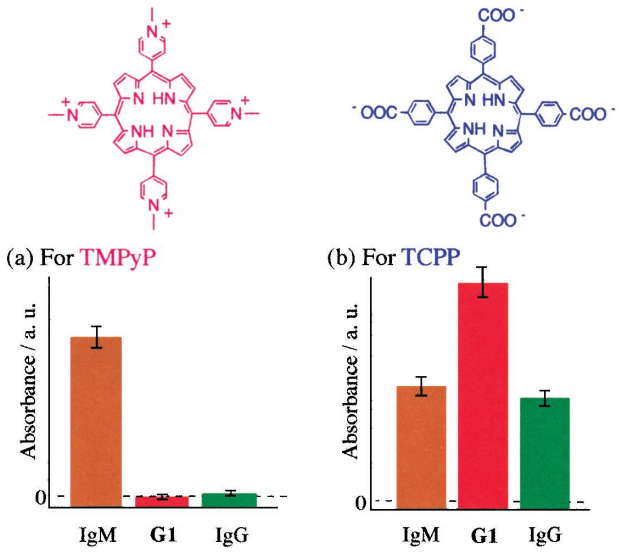


Figure 1. Binding properties of IgG, IgM, and the antibody dendrimer (**G1**) with the cationic porphyrin (TMPyP, a) and those with the anionic porphyrin (TCPP, b) estimated by ELISA.

binding properties of the IgG, IgM, and **G1** with the cationic porphyrin, *meso*-tetrakis(4-methylpyridyl)porphine (TMPyP). Although IgG did not bind the cationic porphyrin and IgM bound the cationic porphyrin, the dendrimer did not bind TMPyP. These results show that the cationic porphyrin attached to IgG occupies the binding sites of IgM in the dendrimer, thus there are no free binding sites against TMPyP on IgM. Figure 1(b) shows the binding of IgG, IgM, and **G1** to TCPP. The IgM used in this study can bind both anionic- and cationic-porphyrins, due to the low specificity of IgM against porphyrins. Both IgM and IgG bound TCPP, while **G1** bound TCPP more efficiently than IgM or IgG. The increase in affinity of **G1** for the anionic porphyrin indicates

that many IgG molecules attach to the surface of the IgM molecule.

The biosensor technique based on surface plasmon resonance (SPR)[19] shows that the antibody dendrimer has an advantage of the amplification of detection signals for antigens. A solution of **G1** was added to the sensor chip on which TCPP was pre-coated by the coupling with hexamethylenediamine as a spacer. The total concentration of the antibody was fixed at 0.2 mg/ml (0.1 mg of IgM + 0.1 mg of chemically modified IgG in 1 ml buffer for **G1**). The sensorgram for the binding of the antibody dendrimer to TCPP was compared with that of IgG to TCPP as shown in Figure 2. The signal intensity increased by the injection of the antibody dendrimer was sufficiently larger than that of simple addition of IgG. Taking into account the change of the binding property of the antibody dendrimer for porphyrins with the increase in the amount of bound antibody to the anionic porphyrin on the SPR biosensor, the antibody dendrimer has many IgG molecules successively bound to IgM molecule.

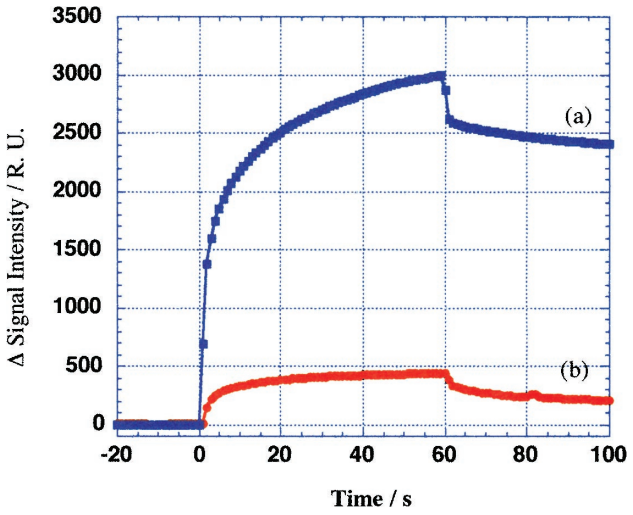


Figure 2. The sensorgrams for the binding of the antibody dendrimer (a) or IgG (b) to the anionic porphyrin immobilized onto the surface of the sensor chip. Phosphate borate buffer (0.1 M, pH 9.0) was used. TCPP was immobilized via hexamethylenediamine spacer onto the sensor chip and then a solution of IgG or the dendrimer was injected to the flow cell. After 60 seconds from the injection of the antibody solutions, flow cell was filled with buffer.

The structural observation of the antibody dendrimer was carried out by using atomic force microscopy (AFM). The sample surface was observed under the suitable conditions that any damage caused by scanning the cantilever is minimized and that any non-specific assembly among antibodies does not occur.[20] Figure 3 shows AFM images of the dendrimer and starting IgM. The image of the dendrimer was twice as large as that of starting IgM. Some branches (IgGs) can be seen outside of the IgM core. Such an assembled structure was not observed in a chemically-modified IgG solution or an IgM solution alone.

In conclusion, new antibody dendrimers were designed and prepared by the combination of IgG and IgM, that is using IgM as a core and IgG as branches. Many binding sites of IgG were

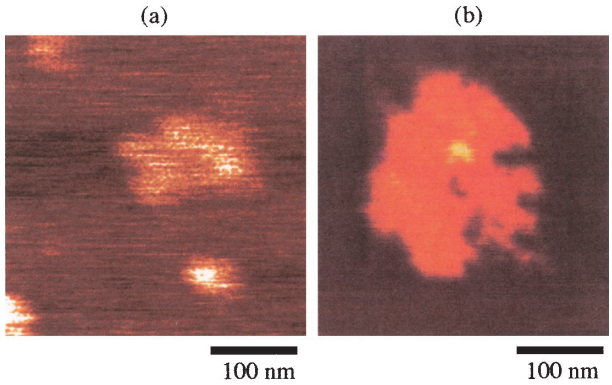


Figure 3. The AFM images of IgM (a) and the antibody dendrimer (b). A total 2 μl of solutions of the antibodies ($3.0 \times 10 - 9$ M) in 0.1 M phosphate borate buffer (pH 9.0) was deposited onto the surface of highly oriented pyrolytic graphite (HOPG) and air-dried.

arranged radially on the surface of one object, the resulting artificial antibodies bound antigens more selective than IgM and more strongly than IgG. The characteristic features of the antibody dendrimer are (i) composed by proteins, (ii) with large size with molecular weight of about 2 million, (iii) constructed by non-covalent bonds, and (iv) binding antigen strongly with high specificity. The antibody dendrimer will be used as functionalized materials for sensitive detection of many kinds of chemicals, for diagnosis, and for drug delivery system.

References

1 J. M. McDonnell, *Curr. Opin. Chem. Biol.*, **5**, 572 (2001).
2 F. W. Scheller, U. Wollenberger, A. Warsinke, and F. Lisdat, *Curr. Opin. Biotechnol.*, **12**, 35 (2001).
3 C. A. K. Borrebaeck, *Immunol. Today*, **21**, 379 (2000).
4 D. Stoll, M. F. Templin, M. Schrenk, P. C. Traub, C. F. Vöhringer, and T. O. Joos, *Front. Biosci.*, **7**, C13 (2002).
5 Y. Kaneda, *Adv. Drug Delivery Rev.*, **43**, 197 (2000).
6 M. C. Garnett, *Adv. Drug Delivery Rev.*, **53**, 171 (2001).
7 B. Golinelli-Pimpaneau, *Curr. Opin. Struct. Biol.*, **10**, 697 (2000).
8 M. C. Feiters, A. E. Rowan, and R. J. M. Nolte, *Chem. Soc. Rev.*, **29**, 375 (2000).
9 Y. Lu, S. M. Berry, and T. D. Pfister, *Chem. Rev.*, **101**, 3047 (2001).
10 W. Shenton, S. A. Davies, and S. Mann, *Adv. Mater.*, **11**, 449 (1999).
11 W. D. Terry, B. W. Matthews, and D. R. Davies, *Nature*, **220**, 239 (1968).
12 E. W. Silverton, M. A. Navia, and D. R. Davies, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 5140 (1977).
13 J. Darnell, H. Lodish, and D. Baltimore, "Molecular Cell Biology," Scientific American Books, New York (1986).
14 H. Yamaguchi, A. Harada, and M. Kamachi, *React. Funct. Polym.*, **37**, 245 (1998).
15 D. A. Tomalia, A. M. Naylor, and W. A. Goggard, III, *Angew. Chem., Int. Ed. Engl.*, **29**, 138 (1990).
16 A. Harada, K. Okamoto, M. Kamachi, T. Honda, and T. Miwatani, *Chem. Lett.*, **1990**, 919.
17 A. Harada, K. Shiotsuki, H. Fukushima, H. Yamaguchi, and M. Kamachi, *Inorg. Chem.*, **34**, 1070 (1995).
18 A. Harada, H. Yamaguchi, K. Okamoto, H. Fukushima, K. Shiotsuki, and M. Kamachi, *Photochem. Photobiol.*, **70**, 298 (1999).
19 H. Yamaguchi and A. Harada, *Chem. Lett.*, **2002**, 382.
20 A. Harada, H. Yamaguchi, and M. Kamachi, *Chem. Lett.*, **1997**, 1141.