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From Fundamentals to Applications

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Preface

"Molecular imprinting" is a newly developed methodology which provides molecular assemblies of desired structures and properties. In the presence of a template molecule, functional monomers are polymerized and immobilized complementarily to this molecule. After the polymerization, the template is removed. During these procedures, a snapshot of the system is taken so that the resultant molecular assembly exclusively binds this template as well as its analogues. This is a kind of challenge towards the second law of thermodynamics saying that "the entropy of the universe should necessarily increase". No one doubts that this method is one of the keys for future science and technology.

The term "imprinting" is reminiscent of psychological phenomena in nature. A newly-hatched chick of some birds (e.g., wild duck) gets an overwhelmingly strong impact from the object that it encounters first after its birth. Thus, it believes that this newcomer should be its own parent. Anything moving can be this "*a posteriori* parent". Other birds, animals, and even human beings are acceptable. Our "molecular imprinting", which occurs between molecules, is also versatile in scope.

This book is written primarily as a textbook for graduate courses. Accordingly, fundamentals of "molecular imprinting" are described in detail. Even undergraduate students should be able to understand the whole context and have a clear idea on this elegant methodology. Experimental details are presented in many reaction examples so that the readers can repeat these experiments and also use this method for their own research. Furthermore, most important recent progresses are covered in chapters 7 and 8. These parts should be very informative even for advanced-course students and researchers who can overview this rapidly growing area and get valuable hints for their future work.

We should like to thank a number of people who have worked for the development of the "molecular imprinting method". Our sincere appreciation is also extended to the staff members and students in our laboratories for helping with

tables and figures, and to our wives, Mitsuko Komiyama, Emiko Takeuchi, and Maki Asanuma, whose understanding was essential. However, as usual, the final criticism must be borne by us.

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Chapter 1
Introduction

1.1
Importance of Receptor Molecules in Advanced Science and Technology

In solutions and gases (but not solids), we know that most of the molecules are randomly moving around. Each molecule does not much care about its neighbors and behaves as it wishes. Complexes between the molecules are formed only through accidental encounters between them. The lifetimes of these collision complexes are negligibly small, and their concentrations in solutions (or in gases) are virtually nil. However, some kinds of molecules (»receptor molecules« or simply »receptors«) precisely differentiate between one molecule and another. They exclusively pick out their own partner molecule from a number of molecules in the system and form a non-covalent complex with this molecule. These complexes are sufficiently stable, and their equilibrium concentrations are considerable. All the molecules other than the partner are completely neglected here, just as we easily and precisely find our friend even in a crowd of people at the station entrance and go to dinner with him or her. When necessary, predetermined reactions and/or catalyses take place in these non-covalent complexes, as observed in enzymatic reactions. Such discrimination between molecules is called »molecular recognition«, and is one of the essential keys to the existence of living things [1].

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In the latest science and technology, the importance of »receptors« and »molecular recognition« has been growing rapidly. This is mainly because one molecule is now a functional unit and has its own role. Highly complicated operations are achieved by a combination of the functions of each molecule. In order to develop highly sophisticated systems under these conditions, we should align a number of molecules in a predetermined manner and allow each to perform its own function. Here, of course, all the molecules must know who are their neighbors, what physicochemical properties they have, and what these neighbors are doing at any moment. This situation is entirely different from ones where bulk properties of materials, rather than the functions of each molecule therein, were the main concerns.

A »molecular imprinting method« has recently been developed to provide versatile receptors efficiently and economically. In principle, movements of molecules are frozen in polymeric structures so that they are immobilized in a desired fashion. This method is so unique and challenging that the scope of future applications is hard to predict precisely at the present time. This chapter deals with the current status of relevant sciences so that the reader can confirm the importance of the molecular imprinting method.

1.2
Naturally Occurring Receptors

There are many cells and molecules in our body, and all of them are cooperatively working in an enormously ordered fashion. Without such mutual understanding and cooperation, we cannot survive. Thus, molecular recognition is essential for the existence of life. For example, the receptors on the surface of cell membranes bind hormones and are responsible for cell-to-cell communication. When the receptor binds a hormone, its conformation is changed and the message of the hormone (e.g., lack of glucose in the body) is passed to the cell in terms of this conformational change. Now that this cell knows what is required in the body at that moment, it promotes (or suppresses) the corresponding

bioreaction(s) to respond to this requirement appropriately. In the above example, glycogen is hydrolyzed and glucose is supplied to the body. The most important thing in these systems is that one receptor accepts only one specific hormone and never significantly interacts with others. Furthermore, this receptor/hormone interaction is enormously strong. Thus, even small amount of hormone can correctly deliver its information to the target cell without information cross talk between cells. On the other hand, selective guest binding by antibodies is essential for our immune response. These proteins patrol around in our body like policemen, arrest a foreign substance (antigen) when it enters the body, and take it to a lysosome (a cell organelle) where the antigen is destroyed. Our body is successfully protected. As would be expected, the differentiation by an antibody between the target antigen and the others (and also between foreign substances and the intrinsic ones in our body) must be rigorously strict.

As is well known, enzymes also show high substrate specificity. Each of them exclusively chooses a certain substrate (specific substrate) and transforms it into a predetermined product. All other compounds in the system (even if they resemble the specific substrate) are kept intact. Another enzyme takes another specific substrate and executes a different mission. This substrate specificity primarily comes from selective guest binding by substrate-binding sites of enzymes. Furthermore, only the specific substrate is efficiently transformed into the desired products, since the catalytically active amino acid residues of enzymes, located near the substrate-binding sites, are arranged suitably only for this transformation.

Detailed information on molecular recognition in nature is now available from X-ray crystallography and NMR spectroscopy [3]. The substrate-binding sites of enzymes are apolar pockets or clefts, which are formed from a number of amino acid residues. There, several functional groups (OH, NH_2, COOH, imidazole, main-chain amide groups, and others) are precisely placed to interact with the functional groups of a specific substrate. For example, an ammonium ion of an enzyme shows Coulomb interaction with a negatively charged carboxylate of its specific substrate. A hydrogen bond is formed between the OH

residues of the enzyme and the substrate. Furthermore, apolar binding occurs between them. For the specific substrate only, all these interactions satisfactorily and cooperatively operate, and a stable non-covalent complex is formed. Antibody-antigen interactions, as well as guest binding by membrane-receptors, occur essentially in the same manner. A number of amino acid residues of antibodies (or receptors) are oriented complementarily to the functional groups of the target antigens (or hormones). Precise molecular recognition also occurs when proteins interact with each other.

1.3 Artificial Receptors

The elegance of molecular recognition in nature has been spurring many scientists to mimic it. One of the greatest advantages of artificial receptors over naturally occurring ones is freedom of molecular design. Their frameworks are never restricted to proteins, and a variety of skeletons (e.g., carbon chains and fused aromatic rings) can be used. Thus, the stability, flexibility, and other properties are freely modulated according to need. Even functional groups that are not found in nature can be employed in these man-made compounds. Furthermore, when necessary, the activity to response towards outer stimuli (photo-irradiation, pH change, electric field, and others) can be provided by using appropriate functional groups. The spectrum of functions is far wider than that of naturally occurring ones.

Pioneering works by Cram, Lehn, and Pedersen (Nobel Prize winners in 1987) established that the following factors are necessary for accurate molecular recognition [1].

1. Functional residues of guest and receptor must be complementary to each other.
2. Conformational freedom of both components should be minimized.
3. Chemical circumstances should be appropriately regulated.

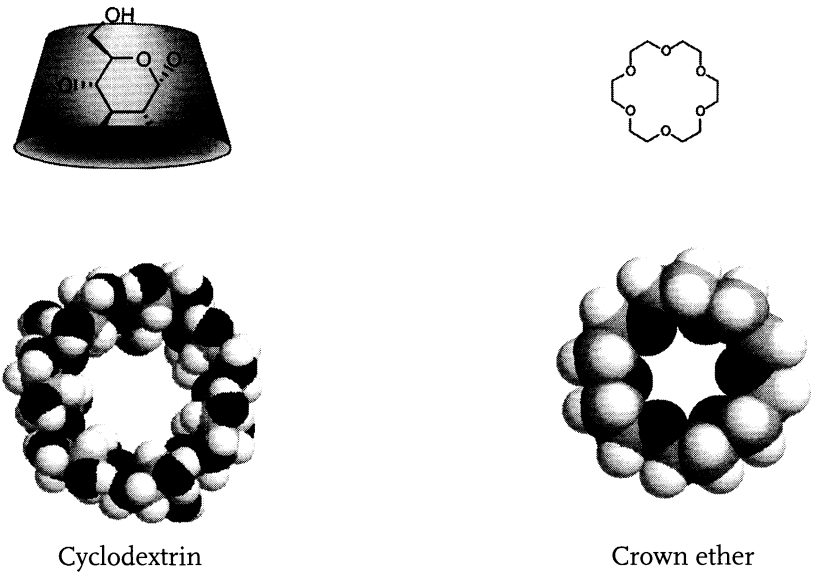

Fig. 1-1 Typical cyclic host molecules used as scaffolds for the recognition of specific target guests

In many cases, various functional residues are covalently attached to cyclic host molecules (e.g., cyclodextrins and crown ethers; see Fig. 1.1). Although each of the interactions (hydrogen-bonding, electrostatic, and apolar-binding) is rather weak, remarkably high selectivities and binding strengths are accomplished when all of them work cooperatively. Alternatively, functional residues are bound to carbon skeletons so that they converge to the central guest binding portion. A typical example of this type of host molecules is presented in Fig. 1.2. In principle, good receptors can be successfully synthesized, as long as (i) we are not concerned about cost and time for their preparation, (ii) our target guest is fairly small, and (iii) we can use organic solvents for the guest recognition. However, these conditions are hardly ever fulfilled, making the molecular imprinting method significant and attractive.

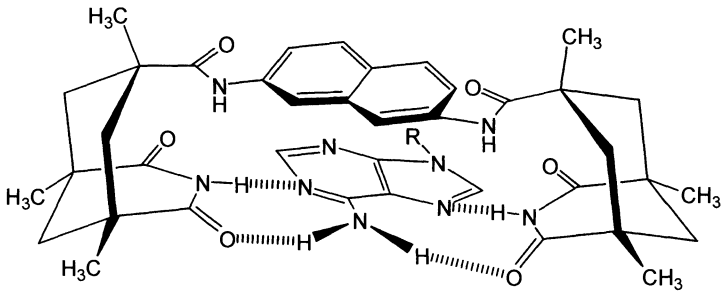

Fig. 1-2 Convergent host molecule for the recognition of adenine derivative [4]

1.4 Receptors for Practical Applications

In industry, receptors are being used to separate the target product economically from reaction mixtures and to remove dangerous chemicals from waste-water. Applications to molecular biology (regulation of bioreactions, separation of biomaterials, and others) are also promising. In some cases, the cost of the separation of the product and its purification accounts for more than half of the total cost of production. Thus, highly selective and economical receptors are crucially important for successful business. Furthermore, unprecedentedly compact and sophisticated devices are fabricated by placing molecules in an ordered fashion. Here, precise recognition between molecules is essential. Molecular memories, molecular devices, and molecular machines have been already realized to some extent [2]. It is also said that artificial cells could be prepared in the near future by placing man-made receptors on artificial cell membranes.

When we design new receptors for future applications, the following factors must be carefully considered [5]:

1. Easy preparation in large amounts at low cost.
2. Stability and activity under wide operation conditions.
3. Selective and strong binding toward large guest molecules.
4. Guest binding in water.

The first and the second requirements are trivial. The third one comes from the fact that important molecules in advanced sciences (proteins, nucleic acids, polysaccharides, bioactive chemicals, and others) are usually large. The fourth requirement has been rapidly increasing in importance, since economical, ecological, and environmental restraints are spurring the replacement of organic solvents with water. For biotechnology, of course, the solvent must be water.

1.5 Why is the Molecular Imprinting Method so Promising?

The molecular imprinting method is quite simple and easy to perform in a tailor-made fashion. All we need are functional monomers, templates, solvents, and crosslinking agents. Polymerization is followed by the removal of the template. During these processes, a number of functional monomers are assembled in an orderly fashion and their functional groups are placed at the desired sites in the cavities of desired size. Neither complicated organic synthesis nor complex molecular design is necessary. If you wish to have a receptor toward a certain guest compound, you can simply polymerize appropriate monomer(s) in the presence of this guest compound (or its analog) as the template. For another guest, you can use another combination of functional monomer and template. The corresponding receptor can be at hand almost automatically. Requirements 1 and 2 would seem to be sufficiently fulfilled. Furthermore, significant progress has recently been made in the fulfillment of requirements 3 and 4 (see Chapter 8). This methodology certainly opens the way to further developments in science and technology.

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Chapter 2
Fundamentals of molecular imprinting

2.1
Introduction

As described in Chapter 1 (Section 1.3), a number of elegant receptor molecules have already been synthesized by aligning functional groups on appropriate scaffolds. Typical examples are presented in Figs. 1.1 and 1.2. They have well-defined molecular structures, and satisfactorily show both high selectivity and high binding activity toward the target guest compound. Detailed and fundamental knowledge of molecular design of sophisticated receptors has also been accumulated through these studies. These trends should go on further and provide still more fruitful results. From the viewpoints of practical applications to industry and our daily lives, however, these synthetic receptors have several drawbacks. The first is rather poor availability. Their synthesis often requires five or more reaction steps, and only a small amount (e.g., 1 g) is eventually obtained. Thus, they are usually too expensive for common industrial uses. Secondly, the design of receptors for large guest molecules is quite difficult, since the scaffolds available are in most cases smaller than several angstroms. Under these conditions, it is hard to place two or more functional groups which are located at notably remote sites (e.g., > 10 Å), even for highly skilled organic chemists using the most advanced organic chemistry. Thirdly, it is difficult to provide these synthetic receptors with an appropriate reaction field for precise molecular recognition (without changing the media used). Precise mo-

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lecular recognition in water is especially difficult, since hydrogen bonds, used in the recognition by naturally occurring receptors, are easily broken because of competition with the water. As is observed in the natural receptors, polymeric structures are usually necessary to form hydrogen bonds in bulk water.

The molecular imprinting method is the most promising solution to these problems. Simply by polymerizing appropriate functional monomers in the presence of a template, desired receptors are cheaply prepared in tailor-made fashion and on a kilogram scale (even on a ton scale). Receptors for large templates are also easily obtainable. No complicated organic synthesis is necessary. Furthermore, the chemical circumstances in guest-binding sites are easily regulated by combining appropriate monomers, crosslinking agents, and/or comonomers. These features make molecular imprinting method one of the most attractive methodologies.

2.2 General Principle of Molecular Imprinting

Suppose that a number of functional molecules interact with a template molecule in solution (or in a gas). The interactions are hydrogen-bonding, electrostatic, apolar, and any other non-covalent interactions. Here, these functional molecules are arranged in an orderly manner with respect to each other so that their functional groups are complementary to the template. Then, what happens if we suddenly remove the template molecule from this system? As you can easily imagine, all the functional molecules would soon start moving randomly, and their ordering would disappear. As a result, the memory about the template is immediately gone. In the molecular imprinting method, however, this randomization is minimized by connecting these functional molecules together by means of a polymer backbone (Fig. 2.1). A kind of snapshot of solution (or gas) is taken, and the structure of the template is memorized in these polymers, providing the target receptors.