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Review Article

Polymers and Gels as Molecular Recognition Agents

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Synthetic polymers and gels capable of molecular recognition are very useful in designing novel intelligent biomaterials. In this article we review the recent progress in both theoretical and experimental studies toward making heteropolymers and gels with biomimetic properties, specifically in relation to protein recognition. Knowledge obtained from protein-folding studies sheds much light on our understanding of the heteropolymer behavior. Consequently, it is possible to design synthetic heteropolymers with specific structure that can fold into unique conformations, form receptor-like cavities and recognize specific target molecules. Recent studies towards simplifying the requirement for the heteropolymer structures and the polymerization procedures are reviewed. Intelligent polymer gels can be designed with new and interesting characteristics of molecular imprinting. The results are encouraging for further investigation and design of synthetic gels with programmable collapsed structure might be achieved.

KEY WORDS: molecular recognition; molecular imprinting; pattern recognition; hydrogels.

INTRODUCTION

In the past few decades biomaterials have evolved from off-the-shelf materials originally developed for applications unrelated to biomedicine to biomaterials specifically designed for particular applications and exhibiting intended biomedical functions (1). The design of new biomaterials requires the combination of knowledge or collaboration among scientists from different fields such as biology, medicine, pharmaceutical sciences, chemistry, physics and materials science. In practice, this new biomaterial development often takes a biomimetic approach by looking into natural processes to examine how the desired functions can be achieved, by understanding these principles and by using them in designing new functional biomaterials (2).

The design and synthesis of polymer materials with ability of molecular recognition can be regarded as the finest exhibit of this biomimetic approach. Molecular recognition can be defined as the ability of a polymer to interact with the designated targets usually amidst a vast range of other molecules, some of which may look almost identical to the target (3). Molecular recognition is ubiquitous and essential in life processes. Examples include enzyme/substrate binding, protein/receptor interactions, and complementary RNA or DNA hybridization (4). These biologic molecular recognition systems have been used widely in designing novel materials such as molecularly imprinted structures (5), tailored colloidal aggregates (6), and biomolecular nanomechanical sensors (7). We will show later that such systems are very promising in advanced pharmaceutical devices.

Synthetic polymers with molecular recognition ability are intriguing biomaterials since they are designed to interact with the biologic environment in a programmable way. For example, a biomedical surface can recognize specific proteins in the biologic fluid, forming complexes with them, and adsorbing a specific protein layer on the surface. In turn, this protein layer may be recognized by the body and trigger a specific response instead of the normal foreign body response induced by the non-specifically adsorbed protein layer on the material surface (8,9).

Another example of this approach is the recently developed class of antigen-responsive hydrogels (10). These hydrogels are semi-interpenetrating polymer networks with complementary antibody and antigen molecules grafted on the polymer. The gels are in their collapsed state (syneresis) due to the extra antigen/antibody complexation, but they swell when put into a buffer solution containing the corresponding free antigens. The underlying mechanism involves free antigens that competitively bind to the antibodies, thus breaking the previously existing interchain complexation and hence triggering gel swelling. These hydrogels possess the ability to recognize the specific antigen and could be used to develop immunoassays and other antigen-sensing materials. A third example is gel formation via hybridization of oligonucleotides (11).

Besides the potential applications in the fields of biology and medicine, the design and synthesis of polymers with molecular recognition is challenging and can fulfill our science ingenuity as pharmaceutical scientists. Indeed, polymer science has reached the point that most of the properties of simple homopolymers have been understood (12–14). Yet, biorelated polymer issues have become one of the foci and points of current interest.

From an applications point of view, numerous biopoly-

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mers have been designed, and studies of their interaction with biomolecules, cells and tissues have attracted significant attention (15). From a scientific point of view, attempts to theoretically model biopolymers and proteins have greatly improved our understanding of structure-property relations of heteropolymers in solutions (16–19). We believe that it is time to apply these principles to design and test of new generation of biomimetic polymers.

The most straightforward way to prepare polymers and gels with molecular recognition ability is to directly immobilize bioactive substances on polymer surfaces, that is to form bio/synthetic hybrid systems (20,21). Thus, the molecular recognition ability of the bioactive substances is transferred to the bio-conjugate polymers. Examples include: lectin-loaded gels to recognize saccharide (22); poly(9-vinyl adenine)-conjugated gels to recognize oligodeoxynucleotides (23); enzyme-conjugated polymers (24); antibody fragment-conjugated gels (25); and streptavidin-conjugated polymers to recognize biotin (26). Bioconjugation as a method to prepare polymers with molecular recognition ability has been a very successful approach, but it is not the focus of the present review. Excellent critical analyses on this approach describe conjugation methods and applications (21,27).

The focus of this review is the design of purely synthetic polymers and gels with recognition ability. As recognition biomolecules for specific targets do not always exist or are difficult to purify, the synthetic approach may be more versatile. In addition, biomolecules such as enzymes or antibodies may lack long-term stability. Several research groups including ours have studied methods of developing polymer networks with molecular recognition capabilities using the molecular imprinting method (28–35).

Traditional molecular imprinted polymer networks are highly crosslinked. The polymer chains in these usually rigid resins have little freedom for conformation change. This is totally different from its biologic counterparts where the biomolecules such as proteins possess enough mobility (36) and recognition processes usually occur through sequential steps of conformation adaptation (37,38).

However, molecular imprinting does provide a versatile approach to synthesize polymers with ability to recognize targets. The molecular imprinting technology combined with the heteropolymer science may enable us to successfully design new polymers and gels still holding conformational mobility. We can even count on this mobility to achieve the molecular recognition, i.e., second-generation molecular imprinted polymers.

Synthetic Polymers with Recognition Ability

In a most general sense the recognition process may be represented as a chemical reaction (39):

$$P + \sum_{i=1}^{n} T_i \rightarrow P - T_k + \sum_{i \neq k} T_i$$

Here, the polymer P specifically recognizes the target T_k from a mixture of molecules T_i (I = 1, n) and forms a polymer/target complex.

The targets can be classified in three categories according to their size and shape: small molecules with sizes scaling to that of the repeating units of the polymers (such as sugar molecules and amino acids); large molecules or colloidal particles with compact shapes (such as various proteins in their native states); and large molecules with loose shapes (such as synthetic heteropolymers). If the compact molecules or colloidal particles are larger than the characteristic size of the polymers, then the targets are effectively two-dimensional surfaces, and the recognition becomes a process where the polymers selectively bind to (and adsorb on) specific heterogeneous surfaces.

The common property of these targets is that they have a distribution of functional groups. These interactions include hydrophobic, hydrogen-bonding, ionic bonding, ligand/metal, and π-π interactions (33). It is the difference in the distribution of these functional groups on the target surfaces or along the linear chains that make the targets recognizable.

We take enzyme/substrate recognition as an example to illustrate how biopolymers recognize their targets. The specific binding of the substrate to the enzyme is determined by the formation of multiple non-covalent bonds between the enzyme and the substrate (40). The binding site of the enzyme molecules is formed by certain amino acid residues that expose specific functional groups to the environment. These amino acid residues usually are not adjacent to each other in the linear sequence, but are brought to proximity and form the binding site by the folded chain structure. The functional groups in the binding site are arranged precisely in the three-dimensional space and form bonds with the corresponding functional groups at the surfaces of the substrate molecules. It is this complementary three-dimensional distribution of functional groups between the enzyme binding site and the substrate that enables the specific enzyme/substrate binding.

The bonds involved in this process are usually ionic, hydrophobic, van der Walls-type and hydrogen bond interactions. For example, the binding of uracil to ribonuclease involves the formation of three hydrogen bonds by uracil and two amino acid residues (serine and threonine) of the enzyme. The two amino acid residues are tens of residues apart from each other in the linear sequence. However, in the folded state of the enzyme they form a binding cavity and expose the three hydrogen-bonding active groups at the surface in a spatially precise arrangement, which is complementary to the distribution of the three functional groups in uracil. This change of the function groups or change in their position greatly decreases the binding ability (40).

Consequently, polymers need to form a stable cavity with a definite shape and specific functional groups in an orientation complementary to that of the target to be recognized target (28). This is exactly the goal of the current molecular imprinting research. The functional monomers are self-assembled around the targets (known also as templates). After the crosslinking agent and targets are removed, the cavities are formed with shape, size and distribution of functional groups complementary to the target. The cavity structure is stabilized by the highly crosslinked rigid network.

As mentioned before, this rigid network is only a phenomenological mimic to the biomacromolecules. We wonder if we can benefit from the molecular principles of these biologic macromolecules and design polymers mimicking them in the molecular level.

From a biomaterials point of view, enzymes and other proteins are nothing more than mostly branched heteropolymers made of various types of monomers. The intricate func-

tions of the proteins are enabled by their three-dimensional structures, which in turn are completely specified by their amino acid sequences, i.e., by the primary structure of proteins (41). Under appropriate conditions (solution and temperature), the interactions among these amino acid residues and between the residues and the solvent molecules make the protein chains adopt unique three-dimensional structures. The loss of the conformational entropy is compromised by the gain of interaction energy (17). On the other hand, simple homopolymers cannot form unique three-dimensional structures even in the folded (collapsed) state, since the different collapsed conformations do not have enough energy difference (42). Theoretically, if we can synthesize heteropolymers with correct monomer sequences along the linear chains, they will fold spontaneously into a predictable and stable three-dimensional structure, and hence will exhibit protein-like molecular recognition ability as shown in Figure 1. We know that the amino acid sequences of proteins are the results of the evolution of millions of years, and therefore the relevant question is if can we find a way to synthesize polymers with properties comparable with these natural proteins.

Inverse Protein Folding Problems

The issue in the protein-folding problem is the prediction of the three-dimensional structures of proteins from their one-dimensional amino acid sequences. The design of heteropolymer linear sequences can be regarded as an inverse protein-folding problem (43). This sequence design problem has two levels: (i) how to design the sequence so that the heteropolymer can fold into a unique conformation; and (ii) how to design the sequence so that the unique conformation can be programmed.

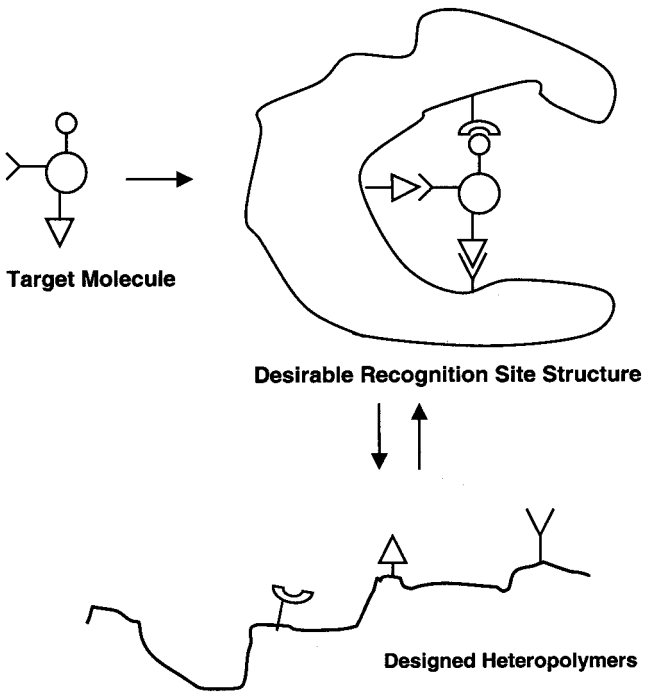

Fig. 1. The spatial structures of the recognition site are determined by the desirable interactions with the target molecules. Through the sequence design, the heteropolymers may fold spontaneously into a stable 3-dimensional structure and form the desirable recognition site.

In recent years we have seen intense interest in the design of protein sequences that can fold into a unique conformation. Using amino acids as monomers (may be not all twenty types) and with the help of computer simulation, numerous *de novo* designed proteins have been synthesized and tested for their ability to fold into a unique ground state conformation (44,45). Of particular importance and impact has been the imaginative and pioneering work that has come out of D. Tirrell's group in the last ten years. For example, Tirrell *et al.* (46) have shown that folded proteinic sequences containing both natural and unnatural aminoacids can be prepared by careful biocatalysis. The ability of these proteins to bind ligand, especially metals, has been tested, and proteins with more complex functions should be expected to be made through this approach in the near future. The ability to incorporate non-natural amino acid analogs into the biosynthesis of proteins has expanded the range of monomers and improved their design ability (46). For example, Urry and his group (47) have used such sequences as innovative biomaterials.

The success of *de novo* designed proteins may not be exciting for the hard-core pharmaceutical and polymer scientists, but provides us confidence to make purely synthetic heteropolymers with recognition capabilities since we know at least we can succeed with heteropolymers as complex as *de novo* designed proteins.

Significant theoretical studies of protein design have resulted in principles helpful for the design of synthetic heteropolymers (48,49). Typically, the desired conformation should be the lowest energy conformation for the heteropolymers with the synthesized sequence, and there should be a large enough energy gap between this desired conformation and other conformations. This energy gap is a necessary condition for the protein stability and for a fast folding.

Another useful conclusion from the theoretical studies is that the effective number of monomer types, m_{eff}, should be larger than the average number of conformations per monomer (49). The parameter m_{eff} is defined as:

$$m_{eff} = \exp\left(-\sum_{i=1}^{m} P_i \ln P_i \right)$$

Here, m is the number of monomer types in the system, and P_i is the probability of the i-th type of monomer represented in the sequence. Therefore, the effective number of monomer types depends on the heterogeneity of the sequence as well as the real number of monomer types. For example, for homopolymers m_{eff} is equal to one, and the homopolymers may possibly fold into a unique conformation only for completely rigid rods.

The average number of conformations per monomer is determined by the flexibility of the chains. The following approaches are suggested to decrease this number (49):

(i) formation of secondary structures along the chain such as α-helix and β-sheet.

(ii) forcing the conformational ensemble of a chain to the set of compact conformations usually achieved by introducing additional non-specific attractions.

We may also apply external constraints to force the chain biased to the compact conformations, as will be discussed later.

De Gennes (50) gave an interesting analysis of the minimum number of amino acid residues required to build up a specific receptor with a folded protein chain. As shown in Fig. 2, to build up a recognition site with n interaction points, the other monomers have to form n-1 loops around the site. After a simplified analysis to the conformation entropy of loops with different lengths, de Gennes estimated that the minimum number of amino acid residues to form such a loop was 13. Therefore, the minimum length of the protein was estimated to be around 13 n amino acids, including n residue form the recognition site. The sequence design is easier than the protein-folding problem since more than one sequence can allow the chains to fold into the desired native state (49).

There has been great progress in the design and synthesis of non-natural oligomers and polymers to emulate the proteins (51,52). Synthetic oligomers have been made and proven to form stable secondary structures (53,54), while the synthesis of polymers with specific sequences has emerged as an exciting field in polymer science (52,55). These heteropolymers are prepared through methods closer to the organic synthesis than polymerization, yet the structures are still too simple and the chains are too short to be claimed as heteropolymers. However, these hetero-oligomers do show promising ability to recognize the intended targets such as metal ions and RNA (51). Breakthroughs in the polymerization methods are expected to help produce heteropolymers with specific sequence, structure and molecular recognition ability.

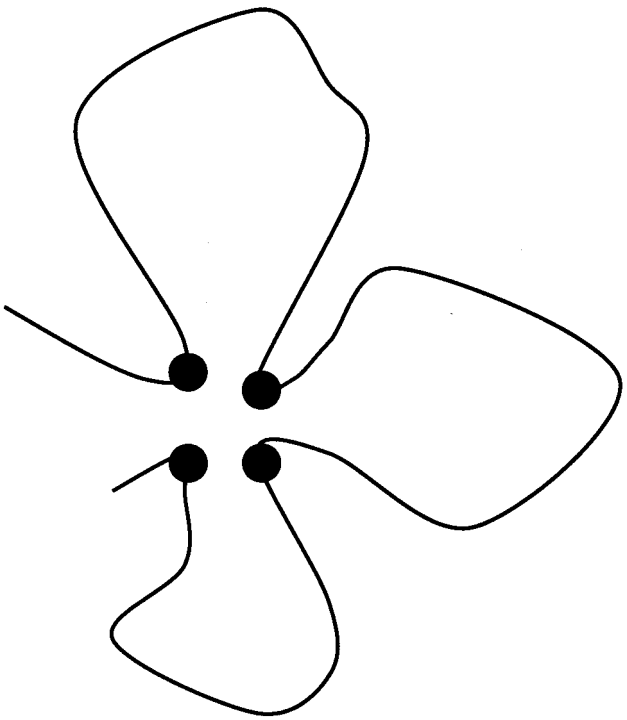

Fig. 2. A schematic illustration of the protein enzyme structure from the polymer view. If a recognition site is formed by amino acid residues of number n (in this figure n = 4), then the whole chain will form n-1 loops around the recognition site. [from de Gennes (50)].

Random Heteropolymers with Molecular Recognition Ability

Compared to the multi-step synthesis of oligomers, our task will become much easier if random heteropolymers can be made to have recognition ability. Though the term randomness does not seem compatible with specific recognition, an integral synthesis and some synthetic "tricks" make this approach rather interesting. For example, Jozefowicz and Jozefonvicz (56) have shown that biospecific molecular recognition can be achieved by random substitution of preformed polymers with suitable chemical groups, or even more simply, by random copolymerization of suitable functional monomer mixtures. For example, they randomly modified dextran polymers with sulphonate, carboxylate, amino acid sulphamide and amide groups, and found that these random heteropolymers bound the complement C3 protein fragment and therefore inhibited the activation of the complement system (56). Similar random heteropolymers have been used (56) to synthesize antithrombin-like polymers, antigen-like polymers, and polymers able to interact specifically with cell membrane receptors.

These apparently surprising results have been explained (56,57) by realizing that the random modification of preformed polymers or the random copolymerization of a monomer mixture results in a family of heteropolymers with multitype chemical groups distributed along the linear chains (see also Fig. 3). A fraction of these polymers with the "correct distribution" of functional groups, which is complementary to the target molecule, will strongly bind to the target, and hence induce the apparent biospecificity. The relative amount of the heteropolymers with the correct sequences should depend strongly on the synthesis condition and relative composition of functional groups, which will result in different distributions of the heteropolymer family.

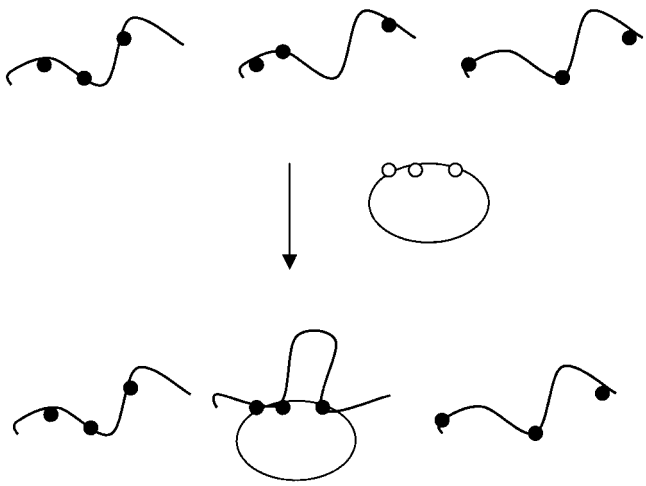

Fig. 3. The random substitution of preformed polymers with suitable chemical groups or the random copolymerization of muti-type monomers yields a family of heteropolymers with various sequence statistics. A fraction of the heteropolymers may have the correct sequence distribution and specifically bind to the target molecules. Selective recognition of random heteropolymers does not require rigid polymer structure. The recognition is not done by the exact shape, but by the match of function group distribution along the chains (56). Its synergistic action with chain conformation changes when interacting with the target molecules.

This random heteropolymer approach can be optimized by choosing the types of monomers or functional groups, their relative compositions, and the backbone chain structure. Further optimization can be achieved by fractionation of the heteropolymer family according to their affinity to the target, using affinity chromatography (57).

This random heteropolymer approach is rather simple and convenient and highlights a different mechanism of molecular recognition. The previously described recognition by the functional cavity formed by folded heteropolymers is traditionally called "lock-and-key" recognition, while the recognition by the flexible heteropolymers is more likely to be described as "induced fit", since the binding will usually induce a conformational change of the flexible heteropolymers. A well-known example of the "induced fit" recognition is recognition of glucose by the enzyme hexokinase (58).

The recognition between complementary single DNA chains is an elegant example showing how flexible heteropolymers can recognize each other; obviously, this is different from the recognition of small molecules or large molecules with compact shapes.

Pattern Recognition by Flexible Random Heteropolymers

To the best of our knowledge, the first theoretical studies regarding the recognition ability of flexible biopolymers can be found in a paper by de Gennes (59). Here, the flexible homopolymer recognition to the print left by another homopolymer was studied in d-dimensional space, where the sites printed by the first polymer exhibited strong but non-selective attraction to the segments of the second polymer segments. This analysis showed that for cases where the dimensionality $d > 2$, the coarse grained image of the imprint could be reproduced by the second polymer, but that the printed sequence would be "read" with the right sequential order only for $d > 4$. The results clearly indicated that in reality two homopolymers would never be able to recognize each other

Further analysis of the recognition capabilities of randomly polymerized heteropolymers may be done by analyzing the polymerization of a mixture of several monomers yielding heteropolymers with statistical sequences, which are determined by the feeding composition and the cross reactivities of different monomers in the reaction mixture (60). "Extreme" examples of these statistical sequences include block and alternate heteropolymers. Generally the statistical sequence can only be described with a set of cross correlation functions $P_i(r, j)$, which can be defined as the probability for a monomer of type-i to have a type-j monomer with the sequence distance of r along the chain.

Chakraborty and coworkers (19,61–65) studied the recognition between these random heteropolymers and multifunctional surfaces. Their studies focus on the adsorption of random heteropolymers (mainly consisting of two types of monomers) with specific sequence distribution from solution on surfaces displaying multifunctional groups according to a statistical pattern. The studies may model the transmembrane signaling process, which is initiated by proteins recognizing a specific pattern of binding sites that constitute a receptor located in a specific part of cell membrane surfaces (19,65). The main question of these studies is if the statistical matching between the polymer sequence distribution and the surface group distribution pattern is sufficient to yield recognition between the heteropolymer and the heterogeneous surface. Using analytical theories and computer simulations, Chakraborty and his associates found that there is a sharp transition from a weak to a strong adsorption after the statistical patterns match each other in a specific way. The sharp transition of the adsorption behavior defines the pattern recognition. More interestingly, after the strong adsorption, the heteropolymers adopt a few dominating pattern-matched conformations.

Dynamic aspects of pattern recognition by flexible heteropolymers have been studied recently (66–71). The recognition process can be differentiated into two steps. In the first step, the heteropolymer binds to the surface but usually not reaching the lowest energy state. In the second step, the adsorbed chain adjusts its conformation, maybe through a complex free energy landscape to reach the perfect bound state, i.e., the so-called "full registration" (71). Though it has been shown thermodynamically feasible, there are still problems left to experimentally realize the pattern recognition by using random heteropolymers.

Molecularly Imprinted Heteropolymers for Recognition

In the past few years, Pande *et al.* (72–75) proposed an interesting approach to use molecular imprinting to synthesize heteropolymers with molecular recognition ability. In a solution of multifunctional monomers and the target molecules, the monomers achieve their equilibrium spatial arrangement according to the heterogeneous interaction among themselves and with the target molecules. In a polymerization method that rapidly polymerizes these monomers in their equilibrium positions, the conformation immediately after polymerization might also be their lowest energy conformation since the same interactions determine the spatial arrangement of the monomers both in the monomer mixture and in the heteropolymers. More interestingly, the heteropolymers in these native conformations form cavities complementary in shape, size and functional group orientation, to the target molecules. Therefore, the cavities are molecularly imprinted by the target molecules. These heteropolymers will thermodynamically reconform into their native conformation, and their cavities may specifically recognize the target molecules (75). This approach seems perfect if it really works. It may be experimentally simple, because it is more like a normal polymerization method and does not involve the complex organic or bioorganic synthesis. More importantly, the sequence design problem and the correlation between the sequence and the cavity structure may be solved by the self-assembly process before polymerization (75).

The approach has been examined using theories and computer simulations (72–75). It was found that in the optimized condition, there is up to 65% success rate that the heteropolymer can renature into its imprinted conformation and form a cavity to recognize the target molecules. We may also use this approach to synthesize target-imprinted heteropolymers which renature to the imprinted conformation only in the presence of the target molecules. Therefore, both "lock-and-key" and "induced fit" types of molecular recognition can be achieved by this approach.

There are no experimental studies to prepare molecularly imprinted heteropolymers using this approach. Yet, in template polymerizations, the monomer units are attached to

the template in a way that is determined by the chemistry and geometry of the template and the monomers, and it is aimed to synthesize polymers or copolymers having microstructures complementary to the template structure (76). Examples of template polymerization include (39) polymerization of acrylic acid (AA)/methacrylic acid (MAA) monomers in the presence of poly(ethylene oxide) (PEO), MAA polymerization in the presence of poly(2-vinyl pyridine), and copolymerization of MAA and styrene in the presence of PEO chains. It has shown that the presence of a linear polymer template has the following effects on the system:

(i) it changes the polymerization kinetics significantly;

(ii) it changes the cross reactivities of the monomers in a copolymerization and the copolymer sequence distribution; and

(iii) it may even change the chemical route of the monomers incorporated into the polymer. For example, the normal polycondensation of urea and formaldehyde in an acid medium leads to the structure -CH_2-NH-CO-NH-, but polymerization with the presence of poly(acrylic acid) chains yield chains with structures like -CH_2-N($CONH_2$)- .

These characteristics of template polymerization are relevant to the molecular imprinted heteropolymer synthesis. We can greatly benefit from studies of template polymerization to experimentally examine the best molecular imprinting approach for heteropolymers with recognition ability.

Polymer Gels with Molecular Recognition Ability

Gels are crosslinked polymer networks swollen in liquids. They are unique materials that can retain their shape and water content. The unique properties of gels make them excellent candidates for numerous applications (77–80). Two characteristics of gels make up the basis for most of their applications: (i) their ability to control the diffusion behavior of molecules in or through them; and (ii) the ability to amplify the microscopic events occurring at the mesh chain level into macroscopic phenomena.

For example, polymer gels may transit between the macroscopically swollen or collapsed states according to slight changes in the environment, which may break the subtle balance among the interactions exerted on the mesh chains, and induce gel phase transition (78). These so-called "intelligent" gels can respond to light (81), temperature (82,83), solution pH (84), magnetic field (85), radiation (86), solvent composition (87), electric field (88), stress (89), and existence of specific molecules in the solution (10,90), the last enabling them to recognize specific molecules.

Figure 4 shows a most important technique, molecular imprinting, for achieving patterns of molecular recognition. Based on the formation of complexes between the template (biological compound) and the monomers participating in the reaction, this process can lead to the formation of superior gels with molecular recognition capabilities.

Gel properties have been compared to those of proteins (78,91,92). Proteins may be in their folded compact or expanded random coil conformations depending on the environment conditions such as temperature. The structure and property similarity between proteins and polymer gels can lead to design of synthetic gels with molecular recognition ability. One approach may be using the heteropolymers, each of which is designed with molecular recognition ability, as mesh chains of the polymer gel. Ideally, in the solution the binding of target molecules with the mesh chains will induce conformation changes, which in turn will cause a macroscopic volume transition of the polymer gels. The mesh chains in the gel are crosslinked with each other through their chain ends and they will inevitably interact with each other. These changes in the structure and environment may cause the heteropolymers to lose their recognition ability completely.

Another approach to achieve gels with recognition ability is to design the whole gel (or gel nanoparticles) as a protein. Gels are made of multi-type of monomers, and they have at least two phases: the swollen phase and the collapsed phase (gels with more than two phases have been studies in the literature (91, 92]. However, similar to the proteins, these designed gels will collapse in a programmed way, and form predetermined conformations in the collapsed state. In the collapsed conformation, a few functional groups come together and form a receptor-like recognition site for the target binding (Fig. 5). Compared with the designed linear heteropolymers, the crosslinks in the gels may act as cofactors to help the programmed gel collapse and the target recognition. Compared with the traditional molecular imprinted networks, designed gels have conformational freedom; only the stable folded state is similar to the rigid network.

Similar to the folding of homopolymers, most of the current polymer gels only collapse randomly. Gels able to collapse in a programmed way and form predetermined collapsed structures offer challenging but also rewarding studies. Recently, Tanaka and coworkers (93–96) extended their studies of molecular imprinted heteropolymers, and synthesized molecular imprinted gels with significantly different affinities to the targets in their swollen and collapsed states. These studies may be regarded as preliminary studies toward the design of gels with programmed collapse.

As a typical example of their experiments (93), the target molecules, pyranine-4, have four negatively charged groups and each of them can form complex with one monomer, methacryl-amido-propyl-trimethyl-ammonium chloride (MAPTAC). The other monomer used in their studies is thermosensitive N-isopropylamide (NIPA). The gels are crosslinked with the presence of the target molecules at a temperature higher than the transition temperature of the normal PNIPA. Therefore, in the polymerization each of the target molecule pyranine-4 forms complexes with four monomers MAPTAC, which should be in a specific spatial arrangement after the gel is formed. After releasing the targets, the re-adsorption of the targets in the gels is studied in two different temperatures, and correspondingly the gels are in the swollen and collapsed states due to the existence of NIPA monomers. Thus, the binding affinity of collapsed gels is two orders of magnitude higher than that of the swollen gels.

The use of PNIPA polymers as the main gel components cleverly introduces the ability to control the gel swelling by temperature, and avoids the normal temperature-induced heteropolymer-folding problem, which may be too complex. A similar system was studied by Watanabe *et al.* (97) with gels crosslinked from monomer solutions of NIPA and acrylic acid with or without target molecules norephedrine or adrenaline. Swelling studies were done with both the molecular imprinted gels and the reference gels in solutions consisting of different concentrations of the target molecules. At low temperature

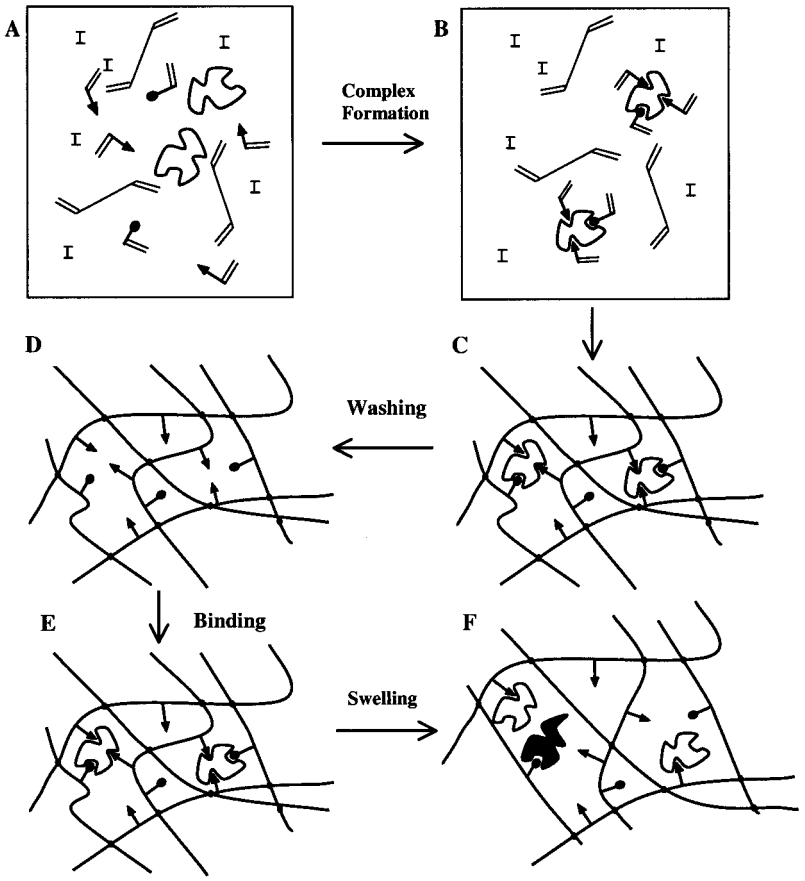

Fig. 4. Imprinting Process. A: Solution mixture of template, functional monomer(s) (triangles and circles), crosslinking monomer, solvent, and initiator (I). B: The prepolymerization complex is formed via covalent or noncovalent chemistry. C: The formation of the network. D: Wash step where original template is removed. E: Rebinding of template. F: In less crosslinked systems, movement of the macromolecular chains will produce areas of differing affinity and specificity (filled molecule is isomer of template).

(the swollen state of the gel), the swelling ratio of both types of gels was independent on the target molecule concentration. However, at higher temperature, the swelling ratio of the imprinted gels increased with the target concentration in the solution, while the reference gels did not show this sensitivity. The results of Watanabe and coworkers are interesting, but the mechanism of this molecular sensitivity has been explained by the macroscopic structures caused by the phase separation in the preparation process, but not related to the memory of molecular conformations (95).

In another interesting study by Nagahori and Nishimura (98), the imprinted gels were made from acrylamide monomers (as the major component) and monomers with sugar units (as the binding component) with/without the presence of lectins concanavalin A (ConA) or *lens culinaris agglutinin* (LCA). It was found that binding affinity of imprinted gels to the corresponding lectins were two order of magnitude higher than those of the reference gels, and the selectivity of the imprinted gels was demonstrated by the cross adsorption experiments with the two lectins.

In our laboratory, we have been working on novel methods of molecular recognition using molecular or nanoimprinting. By analyzing protein-binding domains, we have been successful in designing biomimetic polymer networks that specifically bind biologic molecules in aqueous environment (99). For example, we have synthesized and characterized novel recognition gels for the macromolecular recognition of D-glucose. Further developments will impact applications such as bionanorecognition for diagnostic devices and sensing networks, analyte modulated and controlled drug delivery, drug elimination, and drug targeting (100–102). By tailoring the polymer gel architecture and composition, effective recognition sites were created in polymer gels

These experimental studies seem promising for attracting more attention to design new gels with molecular recognition ability. However, more studies are needed to lead to a major breakthrough.

CONCLUDING REMARKS

Recent progress in heteropolymer and protein folding studies leads to an exciting and challenging field of synthetic polymers and gels with molecular recognition ability. Both the theoretical studies and the preliminary experiments are promising. We expect that collaboration between theorists and experimentalists will lead to molecular recognition comparable to its counterparts in the biologic world.

Theorists will need to offer a unified theory able to de-

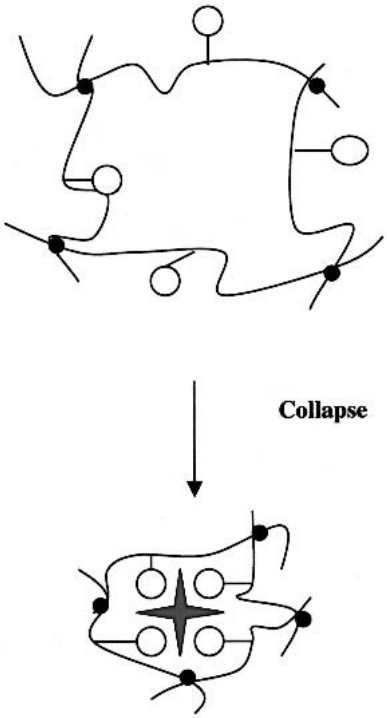

Fig. 5. Gels can be designed to have a programmed collapsed state, where the functional groups come to proximity and form a receptor-like cavity to specifically recognize the target molecules.

scribe and predict the structure-property relation of heteropolymers, their conformation changes and their interaction with other molecules. This theory should be molecular in nature beyond the simple mean-field theory and scaling analysis.

With the progress in the understanding of the heteropolymer structure-property relations, experimentalists should provide novel polymerization methods essential to prepare polymers with controlled linear structure and functional group distributions. Also, we should expect that techniques widely used in biochemistry would find applications to characterize the new heteropolymer systems. The final goal would be to achieve molecularly imprinted heteropolymers and self-assembled structures.

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