Polymer Gels That Memorize Elements of Molecular Conformation

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ABSTRACT: Weakly cross-linked heteropolymer gels that memorize molecular pairs have been designed and synthesized. The polymer consists of a main monomer component responsible for volume phase transition, methacrylic acid that adsorbs one divalent ion as a pair, and cross-links. The memory of pairing of methacrylic acids within the gels was encoded in the primary sequence of main monomers, methacrylic acids and cross-links within the gels, which was achieved by "imprinting", namely, by synthesizing gels while methacrylic monomers were paired prior to polymerization. The control gels, where methacrylic monomers were randomly distributed, showed frustration in forming pairs, whereas such frustration was completely diminished in the imprinted gels allowing the memory of pair formation.

Introduction

Recent theoretical advances in the study of heteropolymers have clarified the major factors that are needed for the memory of conformation in polymers.[1−8] The principles are demonstrated in computer simulations[2,8] and by analytic statistical mechanical theories.[1,3,4] First, heteropolymers, in which the interactions among the monomers have a range of contact energies, can memorize conformation in a condensed state. The heterogeneous interactions create diversity in energies at different conformations, such that some of them can potentially be more favorable than the others. Second, the condensed heteropolymer system connected in a random sequence is frustrated. Because of the interplay of chain connectivity and excluded-volume constraints, formation of some energetically favorable pairs of monomers prevents or hinders the formation of other such pairs. Third, the sequence of the monomer species can be selected or designed to minimize frustration in a certain native conformation.[2] Finally, the minimization of frustration can be achieved by imprinting, namely, by polymerizing monomers while they self-organize in space at a low-energy spatial arrangement.[5,9] The imprinted polymer has the global energy minimum in the original conformation taken upon polymerization. The minimization of frustration is quenched and encoded in the monomer sequence.

The ultimate test of our understanding of these principles lies in the experimental realization of a polymer system that embodies these principles. Recently, polymer systems that memorize partial conformation have been developed. They were heteropolymer gels consisting of major monomer component that allowed for swelling and shrinking of the polymers and minor monomer component that captured target molecules via multiple-point electrostatic interaction, namely multiple number of adsorbing monomers capture one target molecule.[10,11] The polymer networks were randomly copolymerized with only a small amount of permanent cross-links and thiol groups (−SH). The gels were further cross-linked by connecting two thiols into a disulfide bond (−S−S−). These post-cross-links were still in a very low concentrations in the range 0.1−3 mol %, under which condition the polymers could still freely swell and shrink and undergo the volume phase transition. The overall effect of the cross-links, along with the chain connectivity and excluded-volume effects of the polymers, frustrated the adsorbing monomers from coming closer to capture target molecules. To minimize this frustration, the post-cross-links, S−S, were forming while all adsorbing monomers were in complex formation with the target molecules. The "imprinted gels" that were prepared with minimized frustration demonstrated that some of the assemblies of the adsorbing monomers were indeed memorized. In other words, the assemblies were destroyed upon gel swelling, but some of them were restored upon shrinking, re-forming the initial assemblies with the same adsorbing monomers.

This post-cross-linking process, however, has a fundamental drawback. The sequence of the component monomers had already been predetermined and randomly quenched. The minimization of frustration was allowed only in the freedom of finding best partners among SH groups. For this reason the imprinting of conformational memory was only partially successful. Ideally, the entire sequence of all the monomers must be chosen so that the system is in the global energy minimum. This is what has been achieved in this paper.

To demonstrate the general principles for the design and synthesis of gels with the memory of monomer pair assembly, we carefully selected monomer components, cross-links, target molecules, and solvent. Methacrylic acid (MAA) was chosen as the adsorbing monomers with calcium ions as the target. *N*-Isopropylacrylamide (NIPA)

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was used as the major component that allowed swelling and shrinking of the gels in response to temperature. For the imprinted gels, the pair formation of MAA prior to gelation was achieved using lead ions rather than calcium ions because of the strong complexation of $PbMAA_2$ in dioxane, whereas nonimprinted gels were made with MAA.[12−15]

Experimental Section

Design of Polymer Gels. The gels were prepared by free radical polymerization using NIPA (6 M), MAA for nonimprinted, random gels (8−64 mM), or $PbMAA_2$ for imprinted gels (4−32 mM) with cross-linker *N*,*N*′-methylenebis(acrylamide) (BIS, 10−200 mM) in dioxane.[16−19] The dioxane solution of $PbMAA_2$ did not have any conductivity at all, indicating that assemblies of lead methacrylate were intact, which was crucial for the success of imprinting. After the addition of 2,2′-azobis(isobutyronitrile) (AIBN, 10 mM, initiator), the solutions were immediately transferred into a test tube into which glass capillaries of inner diameter of approximately 250 μm were placed. The solutions filled the capillaries and were then degassed under vacuum. The polymerization was carried out at 60 °C for 24 h. After gelation was completed, all the gels were taken out of the capillaries and washed consecutively with deionized water, HCl (100 mM solution), and NaOH (100 mM solution) to remove unreacted molecules and lead ions. The gels were immersed and maintained throughout all subsequent experiments in a 1 mM NaCl solution to ensure complete dissociation of methacrylic acid.

Adsorption of Target Molecules. Pieces of the cylindrical gels of dry weight 40−80 mg were placed in aqueous solutions of $CaCl_2$ (10 mL, 8 μM−0.8 mM). The solution also contained 1 mM NaCl to provide monovalent sodium ions to replace calcium ions. The samples were kept swollen at 25 °C for 48 h or shrunken at 60 °C while being stirred. Equilibrium calcium concentration in the medium was measured using a calcium electrode (97-20 Ionplus, Orion, MA). The amount of calcium adsorbed by the samples was then evaluated by the difference between the initial and the final concentrations.

The adsorption isotherms were analyzed in terms of the Langmuir equation:[20]

$$A = SKC_{eq}/(1 + KC_{eq}) \quad \text{or} \quad C_{eq}/A = 1/SK + C_{eq}/S \quad (1)$$

where A is the amount of calcium adsorbed per unit volume of gel in the collapsed state, C_{eq} is the final equilibrium calcium concentration in the solvent, S is the number of adsorbing sites or the amount of calcium necessary to saturate the adsorbing sites, and K is the affinity of one adsorption site by a calcium ion. From the slope and the intercept at zero C_{eq}, we can deduce both S and K and the overall affinity, SK.

Results and Discussion

The dependence of the parameters S, K, and SK on the concentration of methacrylate groups in the gels in swollen and collapsed states reveals the following conclusions.

1. The adsorption of calcium ions by the gel is well described by the Langmuir's isotherm as formulated in eq 1 (See Figure 1).

2. Figure 2a,b shows that the amount of adsorption sites, S, was approximately equal to half the amount of MAA incorporated in the gels: S = [MAA]/2. This applies to both imprinted and random gels in both shrunken and swollen states. The result of 1 and 2 implies that all the MAA effectively participate in forming adsorption sites for calcium ions when the calcium ion concentration is high enough.

3. In the shrunken state, the adsorption coefficient, K, is proportional to [MAA] for random gels but is almost independent of [MAA] for the imprinted gels

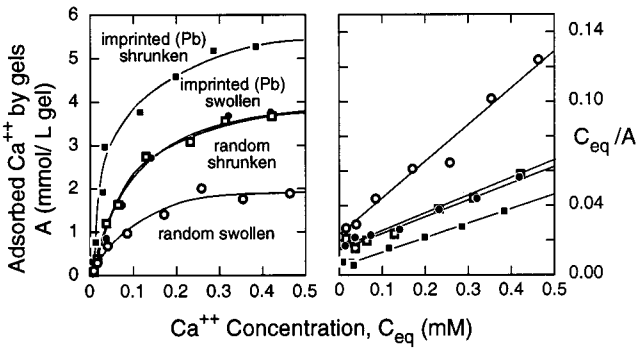

Figure 1. Adsorption of calcium ions by the gels imprinted with lead ions (6 mM $PbMAA_2$) and by the gels randomly polymerized (12 mM MAA) in the shrunken and in the swollen state as a function of calcium chloride concentration. The solution has 1 mM NaCl. The right graph shows the ratio of equilibrium calcium concentration and the adsorption. They become linear with calcium concentration, indicating that the adsorption is well described by the Langmuir isotherm formula.

(Figure 2a). For the gels with 40 mM BIS, the K values for the imprinted and random gels become the same and proportional to [MAA], for MAA above approximately 40 mM. The overall affinity, SK, is proportional to $[MAA]^2$ for the random gels, but for the imprinted gels, is proportional to [MAA]. Above approximately 40 mM of MAA, the SK values for the imprinted and random gels become the same and proportional to $[MAA]^2$.

This result indicates that a pair of MAAs is formed to capture one calcium ion in the shrunken state. In the random gels, the probability for one MAA to find another MAA in its vicinity is proportional to its concentration; thus, $K \propto$ [MAA]. Thus, the probability that two MAAs come into proximity in any given location is proportional to $[MAA]^2$ = [MAA][MAA], leading to $SK \propto [MAA]^2$.

In contrast, in the imprinted gels, each MAA has its original partner MAA nearby, and thus the probability for MAA pair formation is proportional to [MAA] × 1 = [MAA]. The adsorption by the imprinted gels is thus larger than that by the random gels but only up to the concentration of [MAA] comparable to that of the cross-linker concentration, [BIS] = 40 mM. For [MAA] higher than [BIS] each MAA has no frustration and can find a partner nearby. This proves the formation of the pairs of MAA when calcium ions are captured by the imprinted and random gels.

4. In the swollen state, the adsorption by the gels is reduced as reflected in the decrease of K (Figure 2b) and SK (Figure 2c). When the gel is swollen, the distance between the nearest MAAs increases and the probability for pair formation decreases, and K decreases.

5. In the swollen state, the adsorption coefficient, K, is independent of [MAA] for both random and imprinted gels. The affinity, SK, is proportional to [MAA] for both random and imprinted gels (Figure 2b).

As for the dependence on [MAA], simple power relationships cannot be expected between K and SK with [MAA], in the swollen state. As [MAA] increases, the pair formation and, in consequence, K should increase, but at the same time, the volume of the gel rises due to the osmotic pressure of counterions from MAA (e.g., d/d_0 changes from 1.5 for 8 mM MAA to 1.75 for 80 mM MAA). This means that the gel is stretched and becomes less flexible, preventing pair formation. When the gels are shrunken again, the strong adsorp-

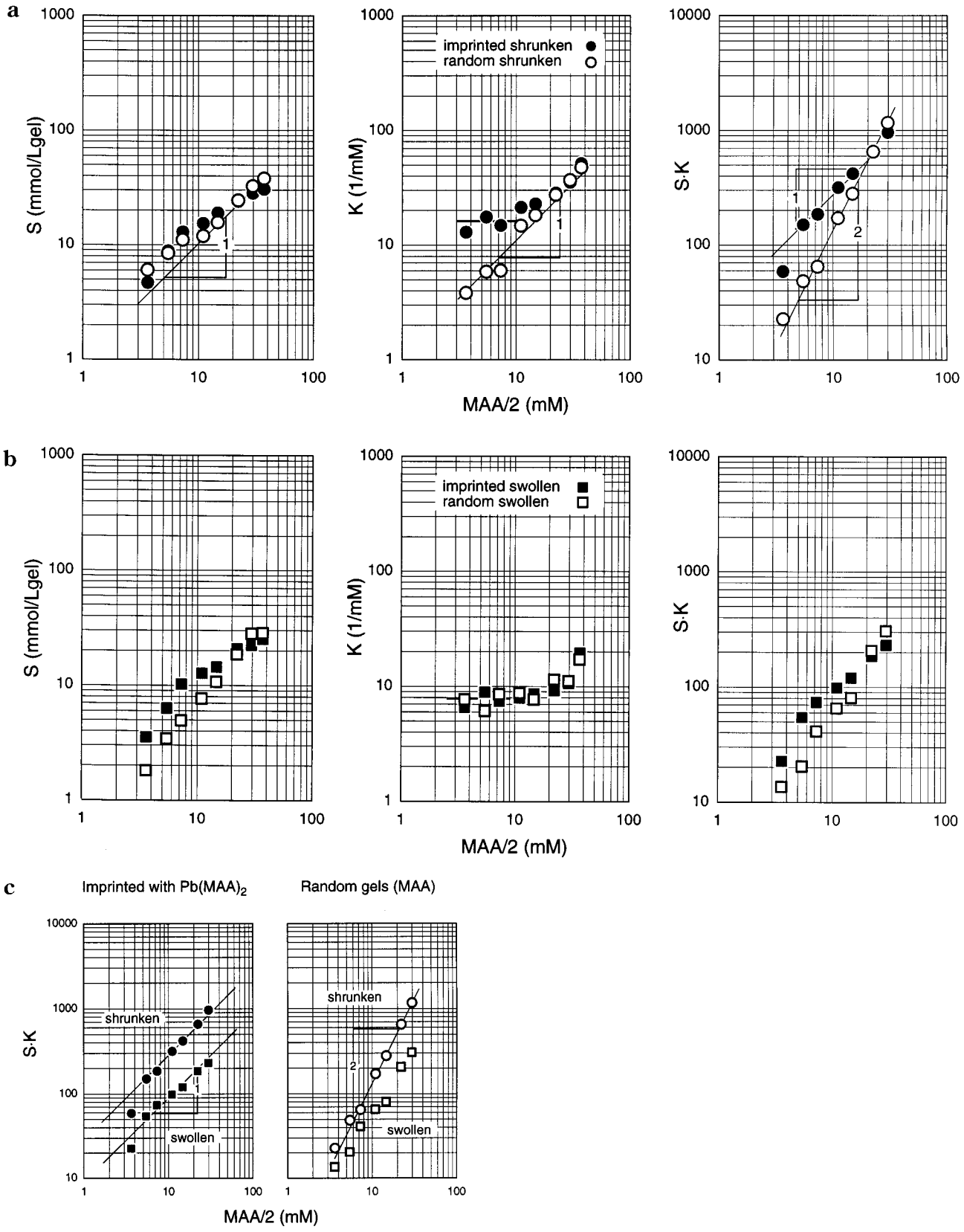

Figure 2. Concentration of adsorption sites within the gels, *S*, and the affinity per adsorption site, *K*, and the overall affinity, *SK*, are plotted as a function of the concentration of the adsorbing monomer, methacrylic acid [MAA]. Cross-link density [BIS] is fixed at 40 mM. Part a is for the shrunken state, part b is for the swollen state, and part c is to show the difference between the shrunken and swollen states. The power laws found between these parameters and [MAA] can be explained as described in the text. The straight lines are theoretical predictions as described in the text.

tion is recovered. This shows the destruction and reformation of calcium adsorption sites made of a pair of MAAs (Figure 2c).

6. Figure 3 shows that, in the shrunken state, the affinity, *SK*, by the nonimprinted gels decays exponentially as a function of cross-link (BIS) concentration. The

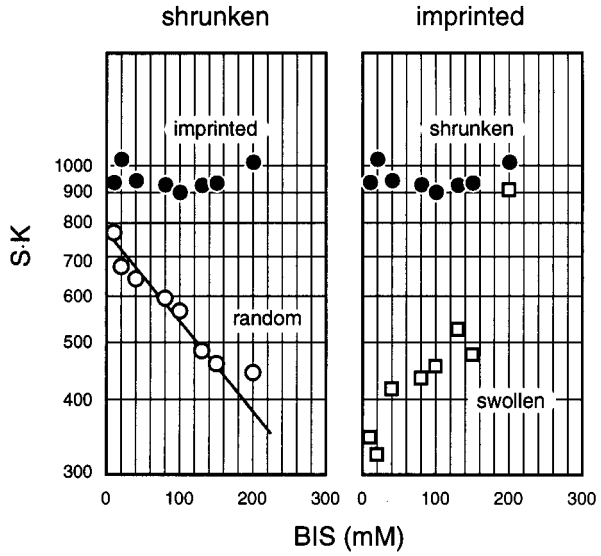

Figure 3. Affinity *SK* to the calcium ions of the nonimprinted and imprinted gels to calcium ions is plotted as a function of cross-link density [BIS] in the left graph. The amount of adsorbing monomers is fixed at [MAA] = 32 mM. The affinity of nonimprinted gels decay exponentially with [BIS] as predicted by the simple statistical mechanical theory of polymers, indicating the frustration, that is, preventing pair formation of MAAs. The frustration is completely diminished by imprinting as shown in the upper curve. The affinity becomes independent of [BIS]. The right graph shows that adsorption is destroyed when the gels are swollen. When high cross-links are used, the gels cannot swell much, and not much difference was observed between swollen and shrunken states.

affinity, *SK*, by the imprinted gels with $PbMAA_2$ does not decrease with BIS.

7. The affinity, *SK*, by the imprinted gels is much larger than that by nonimprinted random gels for 32 mM MAA concentration (Figure 3).

Observations 6 and 7 represent the proof of frustration in the nonimprinted gels and its minimization in the imprinted gels. The affinity *SK* decreases exponentially as a function of cross-link concentration for the nonimprinted gels, whereas for the imprinted gels it is constant.

$$A = A^n_0 \exp(-a[\mathrm{BIS}]) \qquad \text{nonimprinted gels} \quad (2)$$

$$A = A^i_0 \qquad \text{imprinted gels} \quad (3)$$

where a is a constant and A^n_0 and A^i_0 are the adsorption extrapolated to zero cross-links.

The results plotted in Figure 3 can be understood as follows. The MAA units in the gel can move rather freely within a certain volume determined by the cross-link density. Indeed, it is established that, below a certain length scale associated with the cross-link density, the gel behaved like a liquid, allowing the MAA groups to diffuse virtually freely.[16] Beyond that length scale, however, the gel behaves as an elastic solid body, and in particular, the MAA units cannot diffuse beyond the distance. To make a simple estimate, we may assume that each MAA is at one end of a fictitious Gaussian chain with a length half the average polymer length between the nearest BIS cross-links,

$$l = nb = [\mathrm{NIPA}]/[\mathrm{BIS}]/2 \quad (4)$$

Here n is the number of monomer segments of length b. There are $[\mathrm{MAA}]N_\mathrm{A}$ per volume such polymers whose other ends are located in space with an average distance $R = 10\ \mathrm{cm}/([\mathrm{MAA}]N_\mathrm{A})^{1/3}$, where N_A is the Avogadro number. This fictitious Gaussian chain represents the restricted ability of the MAA to diffuse within a certain volume in the gel. We expect that the probability for two MAAs to meet should be proportional to the Boltzmann factor of the entropy loss associated with the formation of one pair of MAAs.

$$P = P_0 \exp(-R^2/nb^2) = P_0 \exp(-c[\mathrm{BIS}]/[\mathrm{MAA}]^{2/3}) \quad (5)$$

Since the adsorption of one calcium ion by two MAAs brings together each end from two fictitious Gaussian polymers, the affinity should be proportional to this probability.

The theory predicts well the exponential decay with [BIS]. Thus, we were able to create frustrations using cross-links and polymerization so that the MAA could not form pairs to capture calcium ions to lower the energy. These results are in agreement with the recent work by Eichenbaum et al. [21] on alkali earth metal binding by nonimprinted (methacrylic acid-*co*-acrylic acid) microgels. They have found that the cross-links prevent the carboxylic groups from achieving the same proximity as in a linear polymer, which affects the binding properties of the metals.

In contrast, there was no dependence of affinity of the imprinted gels on the BIS concentration. This is because the local concentration of MAA in the imprinted gels is very high and is independent of the MAA concentration: $[\mathrm{MAA}] \sim \infty$. Thus, $A = A_0 =$ constant. The imprinted gels have a much larger adsorption than that by random gels because of the minimization of the frustration, and thus the excess adsorption comes from the unfrustrated pairs of MAAs originally paired upon polymerization. If the gel did not memorize such pairs after swelling and reshrinking, an MAA should find a new partner from nearby, and such a probability would be the same as that in a randomly made gel. (Note that upon swelling the affinity is gone.) There would be no difference, then, between the calcium adsorption by the imprinted and the random gels. We can therefore conclude that the excess calcium adsorption by the imprinted gel comes from the successfully memorized pairs.[11,22]

The adsorption by imprinted gels extrapolated to zero BIS is larger than that of the nonimprinted gels. This may reflect the imprinting within linear portions of polymers without the involvement of cross-links.

Conclusions

To summarize, weakly cross-linked polymer gels are prepared consisting of a minority monomer group that can form complexes with calcium ions and a majority monomer group that allows for the volume phase transition of the gel. They adsorb calcium ions upon shrinking and release them upon swelling. The gels are synthesized in two ways, by randomly copolymerizing the monomers or by imprinting, that is, by preforming complexes of carboxyl groups and lead ions and then imprinting the complexes into the polymer network. From the power laws obtained between adsorption parameters and the carboxyl group concentration for both swollen and shrunken phases, we observed that pairs of carboxyl groups are formed to capture calcium ions in the shrunken phase, that the formation is

frustrated in the random gels, and that the frustration is minimized through imprinting. The imprinted complexes are destroyed upon swelling but re-formed upon shrinking. This set of observations constitute a strong evidence for memory of pair formation by a weakly cross-linked polymer network, where the memory is encoded in the primary sequence of majority monomers, minority monomers, and cross-links.

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