

The Volume Phase Transitions of Chemically Cross-Linked DNA Gels Induced by Salts

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A chemically cross-linked DNA gel is demonstrated, for the first time, to be undergone a critical volume phase transition with varying the salt concentration in the solution. The salt concentration at the discrete volume change depends strongly on the valency of the positive salt ion in the solution. For NaCl, the discrete volume change occurs at 4×10^{-2} mol/l for 60% acetone-water mixtures. The MgCl₂ concentration required for discrete volume change are 8×10^{-5} mol/l for 50% acetone-water mixtures, 7×10^{-5} mol/l for 60%. The salt concentration at the volume phase transition is several hundred times larger for monovalent ions than for divalent ions.

Key words : DNA Gel, Volume Phase Transition, Salt

1. INTRODUCTION

An ionized polymer gel undergoes a discrete and reversible volume phase transition with change in temperature, pH and solvent composition^{(1), (2)}. The phase transition is brought about by shrinking of molecules due to tension added to gel, this phenomenon is theoretically predicted by Dusek and Patterson⁽³⁾ based on Flory-Huggins theory in 1968. The theory of first order phase transition induced by collapse of single strand DNA under the condition of random coil was reported in 1979 by Post and Zimm⁽⁴⁾. Recently, a chemically cross-linked DNA gel is found to bring about a volume phase transition upon varying solvent composition^{(5), (6)}. Amiya and Tanaka clearly demonstrated the existence of a first order volume phase transition of natural polymer gels such as DNA, gelatin and agarose gels in acetone-water mixtures⁽⁵⁾. In 1982, Tanaka *et al.* found to undergo a discrete phase transition in equilibrium volume upon varying the salt concentration in the solution⁽⁷⁾. The transition salt concentration depends strongly on the valency of the positive salt ion added to the solution. The gels that have been found to exhibit salt effects on the phase transition of ionic gels so far are all synthetic polymers. The question remains whether discrete phase transition upon varying the salt concentration in the solution is universal for cross-linked polymer gels. In the present study, it is ob-

served that, for the first time, the volume phase transition of chemically cross-linked DNA gel induced by salts. The transition salt concentration for NaCl is several hundred times larger than that of MgCl₂. The salt concentration dependence in the solution on the volume phase transition of chemically cross-linked DNA gel is discussed with ion osmotic pressure.

2. RESULTS AND DISCUSSION

The samples were prepared by standard method⁽⁴⁾. DNA (SIGMA, Type III sodium salt from salmon testes include 5.6% of Na and 12.9% of H₂O) in water (20%) is cross-linked by using ethylene glycol diglycidyl ether (50% of DNA) at pH 11, 55 °C, for 3h. The quantity V_1/V_0 represent the ratio of the final volume to initial volume. All the experiments were carried out at room temperature.

At first, the volume phase transition of the DNA gels in acetone-water mixtures involving NaCl is demonstrated. Figure 1 shows a typical volume phase transition of the DNA gel in acetone-water mixtures involving NaCl under the condition of 1×10^{-2} mol/l NaCl concentration. The discontinuous phase transition of the DNA gel occurs at about 64% acetone concentration. The volume change at the first order phase transition is about 7 times. The transition acetone con-

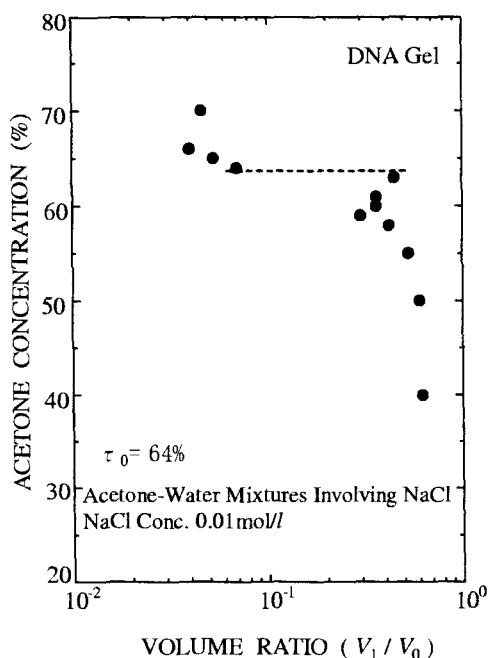


Fig. 1. The volume phase transition of the DNA gel in acetone-water mixtures involving NaCl. The transition acetone concentration τ_0 is about 64% under the condition of gel diameter 2.48mm, and 1×10^{-2} mol/l of NaCl concentration.

centration (τ_0) is changed gently with NaCl concentration range from 1×10^{-5} mol/l to 1×10^{-2} mol/l (τ_0 is 61% for NaCl concentration of 1×10^{-5} mol/l, 59% for 1×10^{-4} mol/l, 59% for 1×10^{-3} mol/l, 60% for 1×10^{-2} mol/l, respectively). The extremely decreasing of transition acetone concentration occurs at NaCl concentration of 1×10^{-1} mol/l. We analyzed experimental results of the dependence of the transition acetone concentration τ_0 on the NaCl concentration, and obtained that varying NaCl concentration brought about the volume phase transition of chemically cross-linked DNA gel in various acetone-water mixtures.

In Fig. 2, a discrete transition of the gel volume occurs at certain critical concentration of NaCl for a given acetone-water mixtures is shown. The first order transition is observed at the NaCl concentration of 4×10^{-2} mol/l for the 60% acetone-water mixtures. The DNA gel is shrunk state completely in the 70% acetone-water mixtures, regardless of the NaCl concentration, since the DNA gel undergoes a collapse at about 64% acetone concentration without NaCl. The volume of DNA gel shows only continuous change in 30%, 40% and 50% acetone-water mixtures. The ethanol-water mixtures also be used to demonstrate the volume phase transition induced by varying NaCl concentration. The discontinuous volume change is observed at the NaCl concentration of 4×10^{-2} mol/l in the 70% ethanol-water mixtures (Fig.3). The

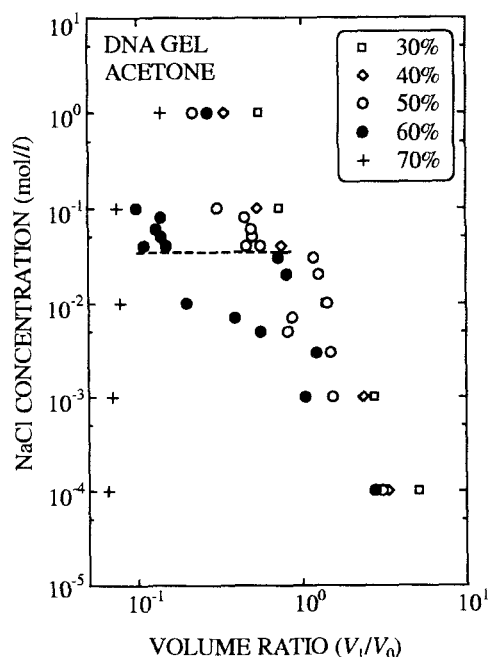


Fig. 2. The swelling volume ratio V_1/V_0 dependence on the NaCl concentrations in 30%, 40%, 50%, 60% and 70% acetone-water mixtures. The transition NaCl concentration is about 4×10^{-2} mol/l for 60% acetone-water mixtures.

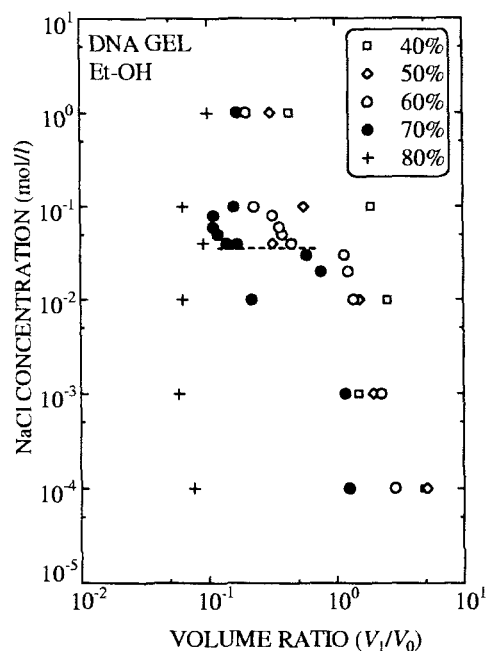


Fig. 3. The swelling volume ratio V_1/V_0 dependence on the NaCl concentrations in 40%, 50%, 60%, 70% and 80% ethanol-water mixtures. The transition NaCl concentration is about 4×10^{-2} mol/l for 70% ethanol-water mixtures.

DNA gel shows only continuous volume change for varying NaCl concentration in 40%, 50% and 60% ethanol-water mixtures, and completely collapsed state in 80% ethanol concentration.

For MgCl₂, the DNA gel shows discrete phase transition at the salt concentrations much lower than for NaCl. The DNA gel required much longer, about five weeks, to attain equilibrium. The volume of DNA gel in 40% acetone-water mixtures shows continuous change upon varying MgCl₂ concentration. The first order phase transitions are observed at 8×10^{-5} mol/l of MgCl₂ concentration for 50% acetone-water mixtures, 7×10^{-5} mol/l for 60%, respectively (Fig.4). For ethanol-water mixture, the volume phase transition is observed at 2×10^{-4} mol/l of MgCl₂ concentration for 50% and 60% ethanol-water mixtures, 3×10^{-5} mol/l for 70%, respectively (Fig.5).

The total osmotic pressure of the gel consists of π_{network} and π_{ion} . network osmotic pressure π_{network} is given by the Flory-Huggins formula. The ion osmotic pressure π_{ion} is defined the sum of the term of counter ion osmotic pressure and the term of the excess decrease of free energy of contact between polymer segments^{(7), (8)}.

$$\pi_{\text{network}} = -\frac{NkT}{v} \left[\phi + \ln(1 - \phi) + \frac{1}{2} \frac{\Delta F}{kT} \phi^2 \right] + \nu kT \left[\frac{1}{2} \frac{\phi}{\phi_0} - \left(\frac{\phi}{\phi_0} \right)^{1/3} \right], \quad (1)$$

$$\pi_{\text{ion}} \equiv f \nu kT \frac{\phi}{\phi_0} + \Delta F_s \frac{N \phi^2}{2v}. \quad (2)$$

Where N is Avogadro's number, k is the Boltzmann constant, T is temperature, v is the molar volume of solvent, ϕ_0 is the volume fraction of the network at the condition the constituent polymer chains have random-walk configurations, ν is the number of constituent chains per unit volume at $\phi = \phi_0$, and ΔF denotes the free energy decrease associated with the formation of contacts between two polymer segments. f is the number of counter ions per effective chain in the gel and ΔF_s the excess decrease of free energy of contact between polymer segments. When the salt concentration is larger than the concentration of the negatively charged groups, the excess decrease of free energy is represented by

$$\Delta F_s = \frac{1}{Z(Z+1)} \left(\frac{n^{*2}}{n_s^0} \right) \frac{\nu kT}{\phi_0^2}, \quad (3)$$

where Z is the valency of positive ions of the salts such as Na⁺, Mg²⁺. Assuming that the network has n^* negatively charged groups (in moles per unit volume) at $\phi = \phi_0$ and n_s^0 is the salt concentration in the solution. For lower salt concentration, ΔF_s may be equals to zero by the analogy of acrylamide gel^{(7), (8)}.

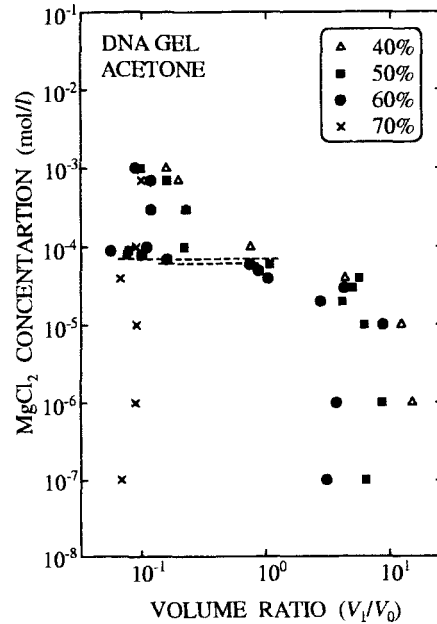


Fig. 4. The swelling volume ratio V_1/V_0 dependence on the MgCl₂ concentrations in 40%, 50%, 60% and 70% acetone-water mixtures. The transition MgCl₂ concentration is about 8×10^{-5} mol/l for 50% acetone-water mixtures, 7×10^{-5} mol/l for 60%, respectively.

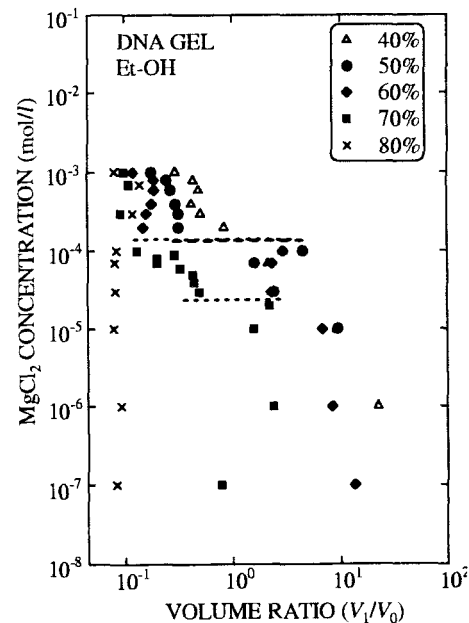


Fig. 5. The swelling volume ratio V_1/V_0 dependence on the MgCl₂ concentrations in 40%, 50%, 60%, 70% and 80% ethanol-water mixtures. The transition MgCl₂ concentration is about 2×10^{-4} mol/l for 50% and 60% ethanol-water mixtures, 3×10^{-5} mol/l for 70%, respectively.

In equilibrium, the osmotic pressure on a gel immersed in a large volume of solvent is equal to zero;

$$\pi_{\text{network}} + \pi_{\text{ion}} = 0 \quad (4)$$

Equation (4) is transformed by Eq.(1) and (2),

$$\begin{aligned} \tau &\equiv 1 - \frac{\Delta F - \Delta F_s}{kT} \\ &= -\frac{v\nu}{N\phi^2} \left[(2f+1) \frac{\phi}{\phi_0} - 2 \left(\frac{\phi}{\phi_0} \right)^{1/3} \right] \\ &\quad + 1 + \frac{2}{\phi} + \frac{2 \ln(1-\phi)}{\phi^2} \quad (5) \end{aligned}$$

The τ is called the reduced temperature, which varies with temperature, solvent composition and salt concentration through the parameter ΔF_s . Within the gel and solvent there are four kinds of mobile ions: H^+ , OH^- , Cl^- and Na^+ or Mg^{2+} . These mobile counter ions in the gel create an excess osmotic pressure. The free energy of contract between polymer segments is changed by increasing salt concentration. For lower NaCl concentration, the network-fixed negative groups is neutralized by mobile counter ions. When NaCl concentration increases and equals to the hydrogen ion concentration, the hydrogen ions in the gel are replaced by the Na^+ . This replacement does not bring about changes in ion osmotic pressure for monovalent ions. But this replacement brings about drastic change in ion osmotic pressure for divalent positive ion. When NaCl concentration equals to that of negative charges fixed on the network, the osmotic pressure of the ions in the solvent increases and the net osmotic pressure of ions decreases. This situation denote that the value of f decrease for the varying salt concentration. At the same time, the reduced temperature τ first increase, and cross the unstable region. Thus, the gel bring about volume phase transition at the optimal NaCl concentration.

In the case of divalent salts such as MgCl_2 , when the hydrogen ions in the gel are replaced by the Mg^{2+} , ion osmotic pressure already drastic change, although not change ion osmotic pressure for the monvalent ion. At the same time, the value of f becomes half value. If the initial reduced temperature is low enough the trajectory cross the unstable region at this $f \rightarrow f/2$ change.

In conclusion, it is demonstrated, for the first time in chemically cross-linked DNA gel, that the universality of the first order volume phase transition is induced by varying salt concentration in the solution.

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