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VOLUME PHASE TRANSITION OF DNA GEL INDUCED BY CATION

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First order volume phase transition of DNA gels induced by the Na (+), Mg (2+), Ethdium Bromide (1+), Hexamine cobalt (3+), spermidine (3+), and spermine (4+) are described to be found to occur, not only as a functions of the concentration in acetone -water mixtures but also in water only.
Key words : DNA Gel, Volume Phase Transition, Cation

1. INTRODUCTION

We wish to report the volume phase transition of DNA gel induced by the positive ions. A chemically cross -linked DNA gel is demonstrated to bring about a volume phase transition with varying the external environments such as temperature, pH and solvent composition. Since Tanaka *et al.* clearly demonstrated the existence of a first order volume phase transition of acrylamide gel [1] and DNA gel cross - linked by EGDE in acetone -water mixtures[2], various polymer gels have been known to exist in two states, swollen and collapsed, like as a first order phase transition. Recently, multiple phase volume transition has been found in copolymer gels consisting of cationic and anionic group, which also form inter - and intra - hydrogen bonding [3].

In order to seek for another biopolymer gels to be revealed on to be involved a multiple phase volume transition as a preliminary experiment, only cation induced chemically cross -linked DNA gel is examined, especially in water solution only.

The volume phase transition is observed in DNA gels with Na^+, Mg^{2+}, Ethidium Bromide (3+), Hexamine Cobalt (3+), Spermidine (3+) and Spermine (4+).

DNA gels were prepared as follows : A fiber in water is cross -linked by using EGDE (50% of DNA) at pH 10~11 at 55℃ for 140 min. stirring and 3 hours keeping in furnace [2].

For Spermidine, the volume phase transition is observed at 2×10^{-4} mol/*l* of Spermidine concentration in water only, and 5×10^{-5} mol/*l* for 60% acetone - water mixtures. On the other hand, for Spermine, the volume phase transition is also observed at 7×10^{-4} mol /*l* of Spermine concentration in water only, and 8×10^{-5} mol/*l* for 60% acetone - water mixtures and also Spermine induces single DNA molecular collapsed [5,6].

The transition point is shifted to the lower concentration as increasing the cation in 60% acetone - water mixtures. The transition valence of concentration for $MgCl_2$ is found to be several hundred times smaller than for the case of NaCl in DNA gel. The salt concentration at the discrete volume change depends strongly upon the valence of the positive salt ions.

2. RESULTS AND DISCUSSION

According to Tanaka [1] for the discussion of polymer gels, the Flory - Huggins formula gives the network osmotic pressure of gel.

$$\Pi = \frac{-NkT}{v}\left[\phi + \ln(1-\phi) + \frac{1}{2}\frac{\Delta F}{kT}\phi^2\right] + \nu kT\left[\frac{1}{2}\frac{\phi}{\phi_o} - \left(\frac{\phi}{\phi_o}\right)^{\frac{1}{3}}\right] + \nu fkT\left(\frac{\phi}{\phi_o}\right) \quad (1)$$

Where N is Avogadoro 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.

In equilibrium, the osmotic pressure on a gel immersed in a large volume of solvent is equal to zero, and then, the free energy of gel, F, is the minimum value. And so, Equation (2) is given, where V is the volume of the gel and $V \propto \Phi_0/\Phi$.

$$\Pi = -\frac{\partial F}{\partial V} \quad (2)$$

In Equation (1), if $\nu v/N\Phi_0{}^3 = 10$, $\Phi_0 = 0.05$ and $\Phi_0/\Phi = V/V_0$, this condition may be expressed as following Equation (3) from Equation (1).

The τ is called the reduced temperature, which varies with temperature, solvent composition and salt concentration. The f is expresses the number of ionization. If f becomes large, the degree of volume phase transition swelling also becomes large.

$$\tau \equiv 1-\frac{\Delta F}{kT}$$

$$=\frac{v\nu}{N\phi^2}\left[(2f+1)\left(\frac{\phi}{\phi o}\right)-2\left(\frac{\phi}{\phi o}\right)^{\frac{1}{3}}\right]$$

$$+1+\frac{2}{\phi}+\frac{2\ln(1-\phi)}{\phi^2}$$

$$=\frac{1}{10}\left[10\left(\frac{V}{V_0}\right)^{\frac{5}{3}}-5\left(\frac{V}{V_0}\right)\left(2f+1\right)-\frac{1}{3}\left(\frac{V_0}{V}\right)\right] \quad (3)$$

DNA gels were prepared by method of Tanaka s group [3]. DNA fiber (SIGMA, Type Ⅲ sodium salt from salmon testes include 5.6% of Na and 12.9% of H_2O) in water (25%) is cross - linked by using EGDE (50% of DNA) at pH 11, 55℃ for 3 hours stirring and 3hours keeping in the furnace. The quantity V_1 / V_0 represent the ratio of the final volume (normally the collapsed state) to initial volume in the swollen state. The ratio is given by $(d_1 / d_0)^3$, where d_0 and d_1 are the initial and final equilibrium diameters of the gel, respectively.

At first, the volume phase transition of the DNA gels induced by NaCl and $MgCl_2$ is demonstrated. The degree of swelling of DNA gel is about 10 to 90 times. The solvent is 60% acetone - water mixture involving Na^+ or Mg^{2+} (three ingredients system). In Fig. 1, the vertical axis (ordinate) shows the solvent composition and the horizontal axis (abscissa) shows the ratio of volume. The volume phase transition is observed at about 1×10^{-2} mol/l of NaCl concentration and about 7 $\times10^{-5}$ mol/l of MgCl concentration for 60% acetone - water mixtures, respectively. The transition concentration for $MgCl_2$ is found to be several hundred times smaller than for the case of NaCl in DNA gel (Fig.1).

In Fig. 2, the volume phase transition of DNA gel induced by Ethidium Bromide in water is shown. An Ethidium Bromide has monovalent cation and intercalate in DNA. An Ethidium Bromide is used staining fluorescence spectrum of base sequence of DNA. There is transition point by about 1×10^{-4} mol/l of Ethidium Bromide concentration for two ingredient system included water not using a hydrophobic solvent. Besides, It seems that DNA gel is melting at near the transition point. This is the first result not using a hydrophobic solvent such as acetone or ethanol. Then it thought that other polyvalence ion could be observed and next, experimented volume phase transition with Hexamine Cobalt, Spermidine and Spermine in two ingredients system included water.

Fig. 3 is the experimental result of volume phase transition using Hexamine Cobalt (3+) as a trivalent cation. A Hexamine Cobalt molecule has a cobalt

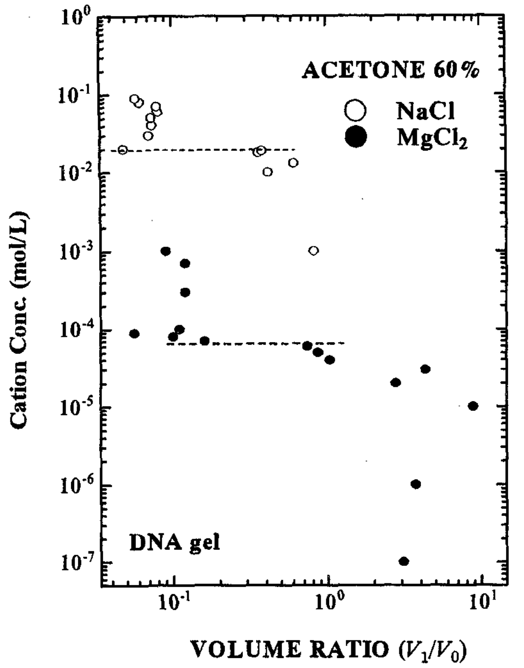

Fig. 1. Volume phase transition of DNA gel as a function of Na^+ and Mg^{2+} ions concentrations in 60% acetone - water mixtures. The volume phase transition is observed at about 1×10^{-2} mol/l of NaCl concentration and about 7×10^{-5} mol/l of MgCl concentration for 60% acetone - water mixtures, respectively.

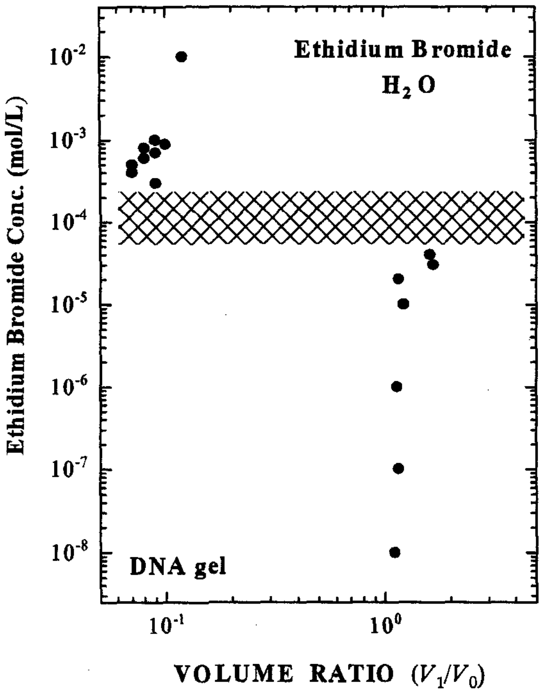

Fig. 2. Volume phase transition of DNA gel as afunction of Ethdium Bromide concentrations in water. The transition Ethdium Bromide concentration is about 1×10^{-4} mol/l for water. It s seems that DNA gel is melting at near the transition point.

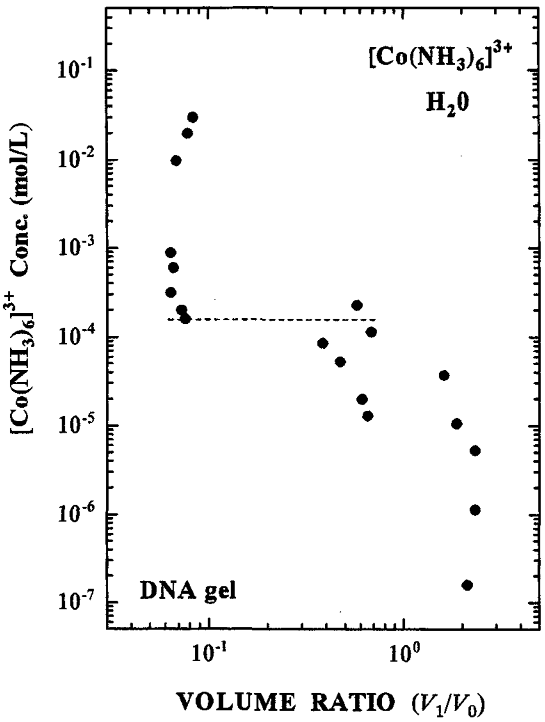

Fig. 3. Volume phase transition of DNA gel as a function of $[Co(NH_3)_6]^{3+}$ concentrations in water. The transition $[Co(NH_3)_6]^{3+}$ concentrations is about 2×10^{-4} mol/*l* for water.

atomat the center, coordinate bonding six amino groups at the around, and it forms complex ion. It s trivalent cation as a whole in aqueous solution. The volume phase transition induced by Hexamine Cobalt is observed in water. The transition $[Co(NH_3)_6]^{3+}$ concentrations is about 2×10^{-4} mol/*l* for water only, and between 1×10^{-5} mol/*l* and 1×10^{-4} mol/*l* for 60% acetone -water mixtures.

Spermidine and Spermine are well known as a DNA unity molecule namely an agglutination of DNA. Spermidine has three amine groups in the carbon skeleton. It s trivalent cation as a whole in aqueous solution. What was used for the experiment is Spermidine - 3HCl of the chloride which dissociates Spermine ions in solution. In water only, the volume phase transition induced by Spermidine is observed. The transition Spermidine concentration is about 2×10^{-4} mol/*l* for water only, and 5×10^{-5} mol/*l* for 60% acetone - water mixtures. Similarly, Spermine has four amine groups in the carbon skeleton. It s tetravalent cation as a whole in aqueous solution. What was used for the experiment is Spermidine -4HCl of the chloride which dissociates Spermine ions in solution. In water only, the volume phase transition induced by Spermine is observed. The transition Spermine concentration is about 7×10^{-4} mol/*l* for water only, and 8×10^{-5} mol/*l* for 60% acetone -water mixtures [3, 4, 5]. The volume change at first order phase transition is about 20 times. This result shows unusual swelling about 5 times near the transition point. This unusual swelling is called melting phenomenon.

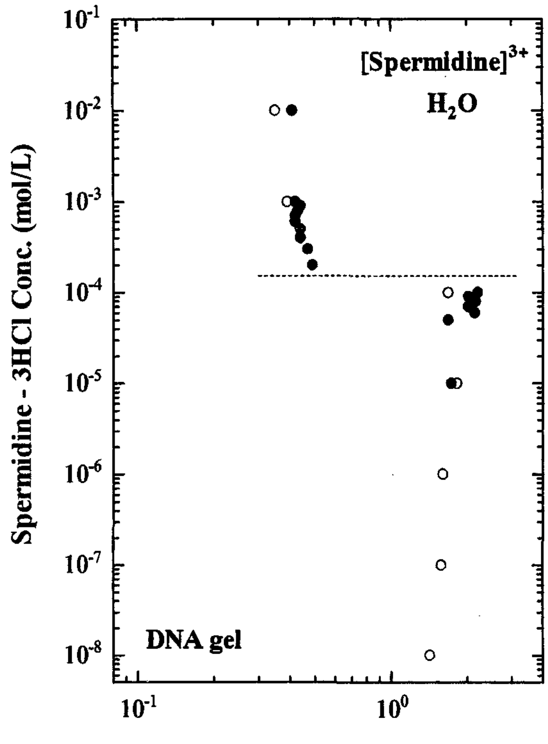

Fig. 4. Volume phase transition of DNA gel as a function of $[Spermidine]^{3+}$ concentrations in water. The transition $[Spermidine]^{3+}$ concentrations is about 2×10^{-4} mol/*l* for water.

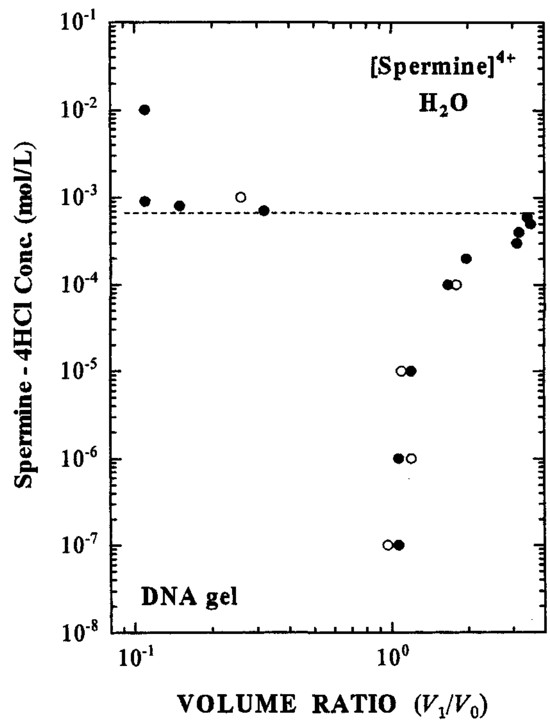

Fig. 5. Volume phase transition of DNA gel as a function of $[Spermine]^{4+}$ concentrations in water. The transition $[Spermine]^{4+}$ concentrations is about 7×10^{-4} mol/*l* for water. It s seems that DNA gel is melting at near the transition point.

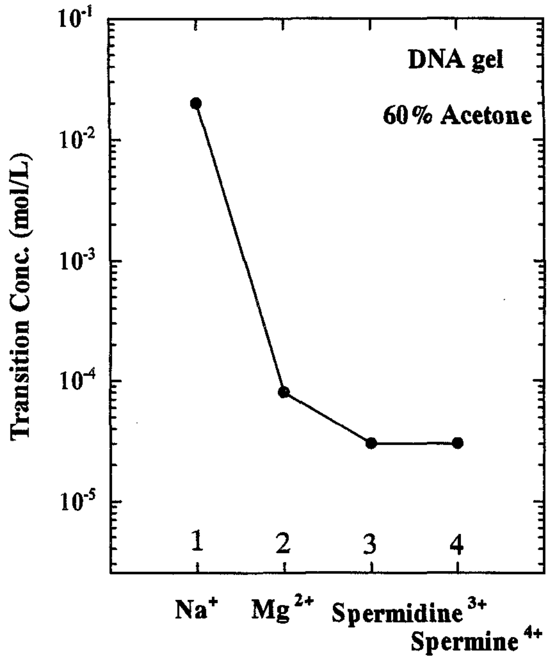

Fig. 6. Transition concentration as a function of valence of cation in 60% acetone -water mixtures. The transition point is shifted to the lower concentration as increasing the cation in 60% acetone -water mixtures. The transition concentration for $MgCl_2$ is found to be several hundred times smaller than foe the case of NaCl.

It is found that the cross - linked between double helix is larger than single stranded DNA by fluorescence spectrum measurement of acridine orange to examine this melting phenomenon. It is suggested that the crosslinking between double helix causes this melting phenomenon. Similar melting phenomenon is observed in the other polyamine and pigment, and suggested relevance the replication of DNA in vivo. The phenomenon comes loose double helix in gene expression.

The mechanism of condensation of single DNA by spemine is shown by the result of computer simulation by Feuerstein, Korolev and *et al.* [6]. That condensation of DNA gel occurred even if the hydrophobic solvent does not exist together is thought that the phenomenon of contraction by the theory of the same coulomb interaction would be occurred also in DNA gel.

The results studied on these cations in DNA gel are as follows. First, volume phase transition of DNA gels are induced by all cations observed. Next, the method of the cross -linked DNA gel is observed between double helix, and the dependency of the valence of cation on the transition point is searched for. When not adding a hydrophobic solvent, the transition point is also decreasing gently with increase as the number of valence of cation. Similarly, the transition point is also shifted to the lower concentration as increasing the cation in 60% acetone -water mixtures. The transition concentration for the cation of bivalence is found to be several hundred times smaller than for the case of monovalence in DNA gel. The salt concentration at the discrete volume change depends strongly upon the valence of the positive salt ions. Finally, first order volume phase transition of DNA gel induced by multi - cations such as Hexamine Cobalt (3+), Spermidine (3+) and Spermine (4+) are revealed not only in 60% acetone -water mixtures but also in water only.

It is summarized that collapse of DNA gel by multi - cations such as Hexamine Cobalt (3+), Spermidine (3+) and Spermine (4+) seems to be induced by the attractive interaction of (PO^-) base of DNA with (NH_3^+) base of cations.

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