行政院國家科學委員會專題研究計畫成果報告

毛細管電泳測量 DNA 之抗衡離子縮合
Capillary Electrophoresis Measurement of Counterion Condensation on DNA

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一、中文摘要

在此計劃中我們利用毛細管膠電泳來測量 DNA 在多價與單價陽離子競爭結合作用後，所導致的電價中和分率。我們發現四種所探討的多價陽離子的電價中和 DNA 的能力大小為：$Co(NH_3)_6^{3+}$ > $spermidine^{3+}$ > Mg^{2+} > Zn^{2+}，所測得的 DNA 電價中和分率隨著其片斷大小增大而增加，並收斂到達一高原值(例如:Mg^{2+}為 0.87)，此時其相對應的 DNA 分子顯然被拉伸並採蛇行方式於高分子溶液中泳動。

在選擇分離介質的實驗中，我們也探討了多種高分子溶液在此 DNA 電價中和測量的適用性。微胞型共聚合物溶液於毛細管壁的吸附性質以及其所兼具的 DNA 電泳分離能力之研究，已經有了具體的成果。

關鍵詞：抗衡離子縮合、電價中和分率、毛細管膠電泳、微胞型共聚合物、

Abstract:

DNA charge neutralization by counterions in the competition binding systems of multivalent cations against monovalent was investigated by using capillary electrophoresis. The charge neutralization abilities of the four multivalent cations studied had the order of $Co(NH_3)_6^{3+}$ > $spermidine^{3+}$ > Mg^{2+} > Zn^{2+}. The measured charge neutralization fractions increased with increasing DNA fragment sizes and converged to a plateau value (e.g., 0.87 for Mg^{2+}) when the electrophoretic behaviors of the corresponding DNA fragments adopted the reptation with stretching mechanism.

In the experiments for selecting DNA separation mediums, we also investigated the applicability of several polymer solutions to our counterion condensation measurements. The results for the studies on the absorption properties of micellar block copolymers on the surface of capillary inner wall and their DNA separation abilities were in press.

Keywords: Counterion condensation, Charge neutralization fraction, Capillary gel electrophoresis, Micellar block copolymer

二、緣由與目的

DNA is a linear polyelectrolyte of high (negative) charge density; thus its conformation is a sensitive function of its ionic environment. In viral capsids, bacterial nucleoids, and chromosomes of higher organisms, DNA occupies about 10^4 to 10^6 times less volume than it does when free in solution [1]. To achieve such a high packing density the strands must be highly ordered, and the repulsive forces between segments have to be overcome. In vivo, DNA packing generally requires proteins and multivalent cations to stabilize the conformation by neutralizing electrostatic repulsions. These DNA condensing molecules include positively

charged proteins such as histones [2], and polyamines such as spermidine and spermine [3]. Schellman and co-workers [4,5] were the first to demonstrate that spermidine^{3-} and spermine^{4+} induced the condensation of DNA in vitro. By using electron microscopy, they found that the condensation product is a well-ordered toroidal structure. Wilson and Bloomfield [6] defined the ionic conditions for DNA condensation by spermidine^{3+} and interpreting their data in terms of the counterion condensation theory developed by Manning [7,8]. They inferred that ~90% of the DNA negative charge must be neutralized for condensation to occur. This value was defined as the critical charge neutralization fraction of DNA condensation. The counterion condensation theory is based on the concept that counterions will condense on a polyelectrolyte to lower its linear charge density to a limiting value. The theoretical formalism describes the territorial (nonspecific) binding of counterions onto a polyelectrolyte as a condensation process which is presumed to occur whenever the ratio of the electrostatic repulsion energy to the thermal energy exceeds the reciprocal of the electrovalence of the counterion. In the presence of a single species of counterion of valence Z, the charge neutralization fraction is predicted to be

$$\theta = 1\text{-}1/Z\xi \qquad (1)$$

where ξ is $q^2/\varepsilon k_B T b$; and q is the proton charge, ε is the dielectric constant of the solvent, k_B is Boltzmann's constant, T is the Kelvin temperature, and b is the average charge spacing along the polyelectrolyte backbone. For DNA in aqueous solution, b = 1.7Å and ξ = 4.2 at 25 °C. The effective charge fraction of DNA in excess M^{Z+}, 1-θ, is 0.24, 0.12, and 0.08 for Z = 1, 2, 3, respectively. In the case that the polyion is in a medium containing two competing counterions of different valence, the theory permits simultaneous calculation of the condensation of each species. For the case of a solution containing univalent counterion of concentration C_1 and Z-valent counterion of concentration C_Z, the effective charge fraction Qf of the polyion due to screening counterions is given by

$$Qf = 1\text{-}(\theta_1 + Z\theta_Z) \qquad (2)$$

where θ_1 and θ_Z are the charge neutralization fractions by the monovalent and Z-valent counterions, respectively, and can be derived from the following set of simultaneous equations (eq 53 and 54 of ref 8):

$$1 + \ln(10^3\theta_1/V_{p1}C_1) = -2\xi(1\text{-}\theta_1\text{-}Z\theta_Z)\ln(1\text{-}e^{-\kappa b}) \qquad (3)$$

$$\ln(\theta_Z/C_Z) = \ln(10^{-3}V_{pZ}/e) + Z\ln(10^3 e\theta_1/V_{p1}C_1) \qquad (4)$$

where κ is the Debye-Huckel constant and e is the base of natural logarithms. V_p is the volume per mole phosphate within which a counterion is considered to be territorially bound.

$$V_p = 4\pi e N_A(1 + Z)(1 - Z^{-1})b^3 \qquad (5)$$

where N_A is Avogadro's number. Manning suggested that the reduction in linear charge density as a result of counterion condensation can be measured by the concomitant reduction in the electrophoretic mobility of the polyion. Many experimental results have been compared and interpreted by the prediction of the counterion condensation theory. As shown by Widom & Baldwin [9] that a trivalent metal ion complex, $Co(NH_3)_6^{3+}$, could also cause DNA condensation when competing with Na^+. The critical charge neutralization fraction obtained was consistent with Manning's prediction. However, they found that $Co(NH_3)_6^{3+}$ was a more efficient condensing agent than spermidine^{3+} and even spermine^{4+}. Some electrophoretic light scattering experiments also showed that counterion condensation theory explained both qualitative and quantitative aspects of DNA charge reduction and conformational condensation, but the predicted electrophoretic mobility magnitude was not yet quantitatively satisfactory [10,11]. Recently conventional slab gel electrophoresis of DNA, in buffers containing mixtures of monovalent and multivalent cations, was used to measure the reduction of mobility of DNA caused by the competition biding of the counterions [12, 13]. In this projet we proposed to use capillary electrophoresis (CE) technique to

study the counterion condensation effect on DNA. CE provides a faster and quantitative measurement for DNA's electrophoretic mobility reduction caused by counterion condensation effect.

三、結果與討論

1. Amphiphilic block copolymers of ethylene oxide, propylene oxide, and butylene oxide can self-assemble to form micelles with different associated structures. At appropriate temperatures and concentrations, polymer networks with DNA sieving ability are formed in various micellar associated structures including closed-packed core-shell micelles, flower-like micelles, and bridged-micelle clusters. Good ds and ss DNA electrophoretic separations can be achieved in the polymer networks with closed or open micellar associated structures. However, these polymer solutions are best for DNA size range of 72 bp to 1353 bp only.
2. An inner-wall treatment of 1N HCl was used to make the capillary inner wall acidic. The micellar block copolymer chains can be adsorbed at the acidic silicon surface so that EOF can be successfully suppressed.

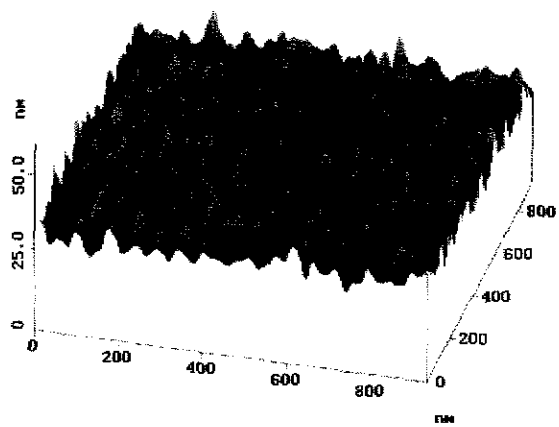

Figure1. AFM image of micellar block copolymers adsorbed on the surface of acid treated silica.

3. The electrophoretic separation conditions chosen from a series of PEO concentrations, electric fields, and capillary lengths resulted in good separation resolutions for all the 8 fragments from λ-DNA and all the 11 fragments from ϕx 174-RF DNA except 271-281 bp.

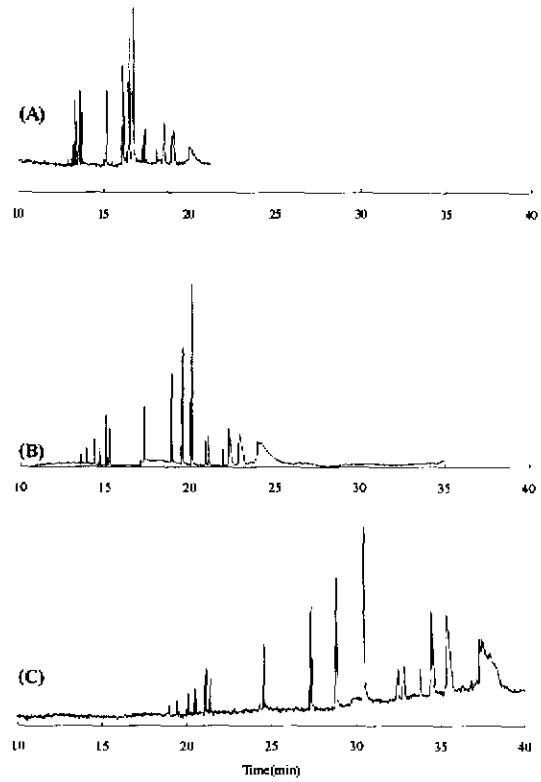

Figure2. Electropherograms obtained for DNA fragments in the size range between 72 to 23130 bp in buffers with (A)[Na^+]=6 μM (B)[Mg^{2+}]=100 μM (C) [Mg^{2+}]=1000 μM, respectively.

4. μ_{DNA} were basically not changed in the 0.5X TB buffer solutions where [Na^+] increased from 6 to 3000 μM. Therefore, we used the averaged μ_{DNA} values for the calculation of charge neutralization fraction.
5. For all the four multivalent cations we studied, μ_{DNA} decreased and thus the charge neutralization fraction increased with increasing amount of added counterion concentrations. As observed in the [Mg^{2+}] system, the neutralization values reached a plateau value of about 0.87, which was in agreement with the prediction of C.C. theory.
6. The charge neutralization abilities of the four multivalent cations studied had the

order of $Co(NH_3)_6^{3+}$ > spermidine^{3+} > Mg^{2+} > Zn^{2+}. spermidine^{3+} was known as a weaker condensing agent for DNA than other conventional 3+ ones. When comparing with Mg^{2+}, Zn^{2+} was much more like the other transition metal ions, which had different preference for binding sites on DNA molecules.

7. The measured neutralization fraction increased with increasing DNA size and had a convergent value when the DNA size was in the region of 2027-23130 bp, where DNA electrophoretic migration behaviors adopted biased reptation mode. In this region, DNA molecules were stretched and fully neutralized.

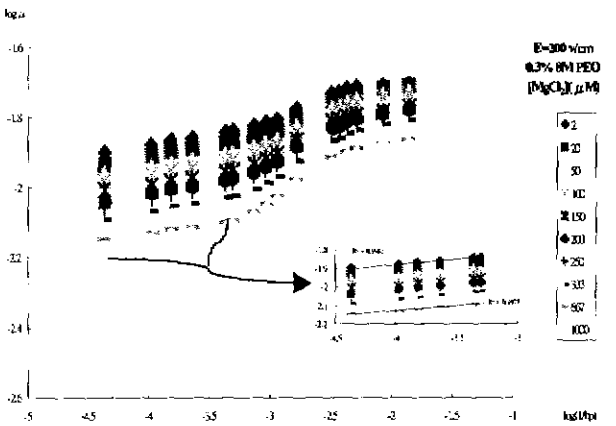

Figure3 . DNAs in the size region of 2027-23130 bp adopted the electrophoretic migration behavior of biased reptation mode.

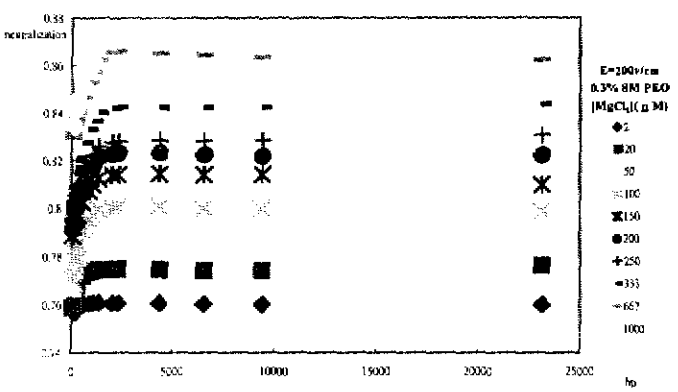

Figure4. Effects of DNA size and [Mg2+] on DNA charge neutralization fractions.

四、計劃成果自評

1. In the experiments for choosing appropriate separation mediums, we found that micellar block copolymers could serve as both capillary column coating materials and good DNA separation mediums. The results of this parts are in press [14] or in preparation.
2. We have provided a fast and easy method for the measurement of DNA charge neutralization fraction caused by counterion condensation effect. This would help us better understand the formation mechanism of the condensed packing structure of DNA in living organisms.

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